

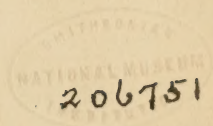
JOURNAL
OF
MORPHOLOGY.

EDITED BY
C. O. WHITMAN,

With the Co-operation of
EDWARD PHELPS ALLIS,
MILWAUKEE.

VOL. X.

BOSTON, U.S.A.:
GINN & COMPANY
1895



CONTENTS OF VOL. X.

No. 1. — January, 1895.

| | PAGES |
|--|---------|
| I. FRANK R. LILLIE. <i>The Embryology of the Unionidae</i> . . . | I-100 |
| II. OLIVER S. STRONG. <i>The Cranial Nerves of Amphibia</i> . . . | 101-230 |
| III. PIERRE A. FISH. <i>The Central Nervous System of Desmo- gnathus Fusca</i> | 231-286 |
| IV. W. K. BROOKS. <i>The Sensory Clubs or Cordyli of Laodice</i> . | 287-304 |
| V. WILLIAM MORTON WHEELER. <i>The Behavior of the Centrosomes in the Fertilized Egg of Myzostoma Glabrum, Leuckart</i> | 305-311 |
| VI. A. D. MEAD. <i>Some Observations on Maturation and Fecundation in Chaetopterus Perga- mentaceus, Cuvier</i> | 313-317 |
| VII. EDMUND B. WILSON AND ALBERT P. MATHEWS. <i>Maturation, Fertilization, and Polarity in the Echinoderm Egg. New Light on the "Quadrille of the Centers"</i> . . . | 319-342 |

No. 2. — February, 1895.

| | PAGES |
|--|---------|
| I. ALBERT C. EYCLESHYMER. <i>The Early Development of Amblystoma,</i> <i>with Observations on Some Other</i> <i>Vertebrates</i> | 343-418 |
| II. T. H. MORGAN. <i>The Formation of the Fish Embryo . . .</i> | 419-472 |
| III. H. F. MOORE. <i>On the Structure of Bimastos Palustris,</i> <i>a New Oligochaete</i> | 473-496 |
| IV. J. PERCY MOORE. <i>The Anatomy of Bdelodrilus Illuminatus,</i> <i>an American Discodrilid</i> | 497-540 |

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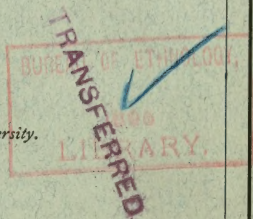
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VOL. X.

JANUARY, 1895.

No. 1.

BOSTON, U.S.A.:
GINN & COMPANY.

AGENT FOR GREAT BRITAIN:
EDWARD ARNOLD,
*37, Bedford Street, Strand,
London, W.C.*

AGENTS FOR GERMANY:
R. FRIEDLÄNDER & SOHN,
*Berlin, N.W.,
Carlstrasse 11.*

AGENT FOR FRANCE:
JULES PEELMAN,
*189, Boulevard Saint-
Germain, Paris.*

CONTENTS OF No. 1, JANUARY, 1895.

| | PAGES |
|--|---------|
| I. <i>The Embryology of the Unionidae.</i> FRANK R. LILLIE | 1-100 |
| II. <i>The Cranial Nerves of Amphibia.</i> OLIVER S. STRONG | 101-230 |
| III. <i>The Central Nervous System of Desmognathus Fusca.</i> PIERRE A. FISH | 231-286 |
| IV. <i>The Sensory Clubs or Cordyli of Laodice.</i> W. K. BROOKS | 287-304 |
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JOURNAL

OF

MORPHOLOGY.

THE EMBRYOLOGY OF THE UNIONIDAE.

A STUDY IN CELL-LINEAGE.

FRANK R. LILLIE.

TABLE OF CONTENTS.

| | PAGE |
|--|------|
| I. INTRODUCTION..... | 2 |
| II. CLEAVAGE OF THE OVUM AND DERIVATION OF THE GERMINAL LAYERS | |
| a. <i>Natural History</i> | 5 |
| b. <i>Methods</i> | 7 |
| c. <i>Nomenclature</i> | 8 |
| d. <i>Cleavage</i> | 10 |
| 1. The First Two Furrows..... | 10 |
| 2. The First Generation of Ectomeres..... | 13 |
| 3. From the Eight to the Seventeen-cell Stage..... | 18 |
| 4. From the Seventeen to the Thirty-eight-cell Stage; Third Generation of Ectomeres, etc..... | 19 |
| 5. Establishment of Bilateral Symmetry..... | 23 |
| 6. The First Somatoblast..... | 24 |
| 7. The Second Somatoblast..... | 27 |
| e. <i>Gastrulation and Shell-gland</i> | 28 |
| f. <i>Summary</i> | 30 |
| g. <i>General Remarks on the Cleavage and Germ-layers</i> | 32 |

| | PAGE |
|---|------|
| III. GASTRULA TO GLOCHIDIUM | 46 |
| a. <i>Gastrula to Young Larva</i> | 46 |
| 1. Ectodermal Organs | 46 |
| 2. Mesoblastic Organs | 54 |
| 3. Entodermal Organs | 58 |
| 4. Summary | 58 |
| b. <i>Transformation of the Young Larva into the Glochidium</i> | 60 |
| c. <i>Axial Relations</i> | 71 |
| IV. APPENDIX | 76 |

THE work recorded in the following pages was begun in the summer of 1891 at the Wood's Holl Marine Biological Laboratory, at the suggestion of Professor C. O. Whitman. It has been pursued since, subject to more or less interruption, at Clark University and the University of Chicago. In each laboratory I profited by the instruction and advice of Professor Whitman. It gives me great pleasure to acknowledge in this place my deep indebtedness to him.

I. INTRODUCTION.

The Unionidae are in many ways extremely favorable objects for embryological research, and in consequence have often been the subject of more or less careful investigation. The great ease of obtaining an almost limitless supply of material, the comparatively large size of the eggs, and the transparency of many of the larval stages make them favorable for embryological work. On the other hand, the early stages of development offer considerable technical difficulties to the student. Moreover, the extremely specialized character of the development seems to promise but little of general morphological interest.

The history of recorded observations on the embryology of the Unionidae is a long story; and as it has been rehearsed more than once by other writers on the subject (Rabl No. 25, Flemming No. 13), I need only say enough here to make my point of departure clear. A list of the early literature on the subject will be found at the end of this paper. The question

which produced such a voluminous discussion (see Part I, list of literature) was in regard to the real nature of the curious little bivalves, which were often found filling the external gills of adult Unios and Anodontas. Were they parasites or were they the young of the animals which they burdened with their incredible numbers? Rathke (No. 7, 1792) and Jacobson (No. 3, 1828) held that they were parasites of a different genus, and proposed for them the name of *Glochidium parasiticum*. The action of the Paris Academy of Sciences, which appointed a commission (No. 4) to inquire into the foundations of this curious view, is sufficient proof of the lively interest which the question excited. It would take me too far to follow the discussion in detail; it will be sufficient to say that Carus (No. 5, 1832) gave the final blow to the glochidium theory in a paper which is remarkable both for accurate observation and logical argument. Indeed, but little advance was made beyond the facts established in this paper prior to Flemming's work (Nos. 12 to 14). Contributions to the subject had been made in the meantime by Quartrefages (Nos. 23 and 24), Leuckart (No. 19), O. Schmidt (No. 20), Forel (No. 21), and van Ihering (No. 22). In 1866 Leydig (No. 20a) made the valuable discovery that the glochidium larva finishes its development as a parasite on fish.

Flemming's principal paper appeared in 1875 (No. 13). He gave some good figures of the maturation and early segmentation stages of the egg, and of the later larval stages; but he remained in the dark as to the passage from the one to the other, and hence came to no definite conclusion as to the origin and formation of the germinal layers. Rabl's paper (No. 25) appeared in 1876, and is in some ways a distinct advance on Flemming's. He endeavored to prove that the germ-layer theory was applicable to the Unionidae "wie bei allen Metazoen." Although he was undoubtedly right in saying that the "Vorderwulst" of Flemming was entodermic, and that the mesoderm arose from two teloblasts, yet he was just as undoubtedly wrong in his account of the origin of the entoderm as well as of the teloblasts of the mesoderm. According to Rabl, the entoderm arose as an invagination of large cells in

the region of the future dorsal surface, which freed themselves after invagination and moved to the anterior end (really to the posterior end) of the body, where they took up their definite position. Believing, as he did, that the dorsal invagination was the primitive intestine, it was natural to suppose that the teloblasts of the mesoderm, which lie just beneath the posterior end of this invagination, were derived from the cells composing its wall. There was a threefold error here: first, in mistaking the posterior for the anterior end of the larva;¹ second, in interpreting the dorsal invagination, really the shell-gland, as the archenteron; and third, in deriving the teloblasts of the mesoderm from the cells of the dorsal invagination. The first error was corrected by Schierholz (Nos. 35 and 36), and the second by Goette (No. 29). In my preliminary account I have already pointed out the true source of origin of the teloblasts of the mesoderm.

Not since the time of Rabl's paper has any work been published dealing in a thorough way with the whole embryonic development of the glochidium larva. Schierholz's paper, it is true, covers the whole period of development, but does not adduce much that is new on the embryonic portion other than the correct orientation of the larva. Goette's paper confines itself to the formation of the entoderm in Anodonta. Other papers since the time of Rabl have dealt only with the postembryonic development, thus beginning where my work ends, and hence not calling for remarks here. It would seem, then, as though it were time for another work on the embryonic development.

Great advances have been made within the last fifteen years in the adult morphology of the Lamellibranchiata; but since the time of Hatschek's paper on *Teredo*, no corresponding advances have been made in the embryology of these forms. In saying this I do not mean to belittle such works as those of Brooks, Jackson and Horst on the oyster or Ziegler and Stauffacher on *Cyclas*. During the same time many important embryological works on other classes of Mollusca have

¹ Flemming made the same mistake; Forel, who in point of time preceded both Flemming and Rabl, was right.

appeared, but, as if by common consent, the Lamellibranchiata have been ignored. The eggs of most marine lamellibranchs are exceedingly small and difficult to handle. This has probably deterred others, as it has me, from studying them, or has made the results of such studies as have appeared (*e.g.*, on the oyster) of greater economic than scientific worth.

My object in studying the Unionidae has been to fill in, so far as I could, the two gaps indicated above, *i.e.*, first, in our knowledge of the derivation of the germinal layers in the Unionidae; and, second, in our knowledge of the early development of the Lamellibranchiata. I have followed in detail the lineage of the cells as the best way of reaching conclusive results as to the origin of the germinal layers of Vermes and Mollusca. The first division of the work deals with the origin of the germinal layers. In the second part I take up the development of the gastrula in the glochidium.

II. CLEAVAGE OF THE OVUM AND DERIVATION OF THE GERMINAL LAYERS.

(a) *Natural History.*

The Unionidae carry their young in the external gills until the completion of embryonic development, when they are fully equipped for their temporary parasitic existence. The eggs are fertilized, after extrusion, in the suprabranchial chamber (Schierholz, No. 30, Rabl, No. 25), spermatozoa gaining access with the respiratory current of water. They then pass along the suprabranchial chamber of the internal gill to the cloaca, where they pass over to the external suprabranchial chamber, and moving along it anteriorly fill the interfoliar spaces from one end of the gill to the other.¹ In *Unio* the eggs adhere to one another in the form of oval plates, which may contain as many as 1000 eggs² each. The eggs composing each plate are bound together by a sort of cement, which gradually dis-

¹ I noticed that, in a species of *Anodonta*, which is very common near Worcester, Mass., only the posterior half or two-thirds of the external gills is used for the reception and development of the ova.

² A rough calculation.

solves out as development proceeds, finally disappearing entirely towards the close of embryonic development. The eggs of *Anodonta* are free from the first.

I obtained most of my material from two ponds within five or six miles of Wood's Holl. In one of these, Chiverick Pond, I obtained *Unio* only; in the other, Fresh Pond, *Anodonta* only; yet the ponds were within 500 or 600 feet of each other. The first pond, however, possessed a softer muddier bottom than the second, which was literally paved near the margin with round stones as large as cobble-stones. No doubt the different sets of conditions proved more favorable to the one genus or the other; but it can hardly be held that conditions most favorable to the one genus were necessarily fatal to the other nearly allied genus. The close proximity of the two ponds in question makes it probable that an interchange of the genera in question takes place at not infrequent intervals. Indeed, I once found a sickly-looking *Anodonta* in the *Unio* pond. It is therefore improbable that this condition was due to a difference in the original stock of the ponds in question. It seems probable that in small bodies of water, at any rate, the two genera *may* prove mutually exclusive.

An interesting difference between these two genera has been commented on by Flemming (No. 13), Schierholz (No. 30), and Carus (No. 11). This is, that, whereas all the *Anodontas* in a given locality extrude their eggs (which are immediately fertilized) at one or two different times, so that from a great many individuals taken at the same time only one or two different stages of development are procured, the different individuals of *Unio* are fertilized at different times, so that a large number taken at one time will yield several different stages of development. It is, however, noticeable in the last case that the embryos of one catch can always be grouped in five or six stages. It is thus much easier to obtain a complete developmental series of *Unio* than of *Anodonta*. For this reason, and also because the eggs of *Unio* are much more favorable for the study of segmentation and for sectioning, I have worked almost exclusively on this form. What I have to contribute on *Anodonta* concerns the glochidium only. The unsegmented

As No. 1 of Vol. X is fully equal in bulk and expense to two ordinary numbers of the Journal, it seems best to close the volume with No. 2. Future volumes will contain, as usual, three numbers.

THE EDITOR.

fertilized ova of *Unio* can be obtained from about the middle of June to the middle of July in the above locality; those of *Anodonta* towards the end of July and early in August.

The glochidia of *Unio* escape in August and September. Those of *Anodonta* are carried by the mother through the winter and extruded finally in the spring. Most remarkably slow is the process of cleavage in these eggs. The passage from the unsegmented egg to the 45-cell stage takes from five to seven days. I have not been able to get exact data in regard to the time of development, inasmuch as it is impossible to keep the same eggs under observation for more than a very few days. They then die, despite the utmost care. I have seen enough, however, to show the extreme slowness with which the eggs develop. Inasmuch as these ova contain but little yolk, we can only refer this slowness in development to constitutional causes. I know of no observations on the rate of development of the ova of *Cyclas*. On the other hand, the ova of marine lamellibranchs develop with amazing rapidity. During warm weather the young of the oyster will swim in less than twenty-four hours after fertilization.

(b) *Methods.*

The vitelline membrane is separated from the ovum by a wide space (Figs. 2 and 3, Pl. I) filled with an albuminous (or mucous) fluid, which coagulates on the addition of the usual killing reagents, making it impossible to obtain clear views of embryos mounted whole. During development the constitution of this fluid alters somewhat, so that this difficulty ceases to exist for stages later than Fig. 79 (Pl. V). I was, however, fortunate enough to find a method which gave me, at times, the most perfectly clear views of segmentation stages that could be desired; at other times it was not so successful. The embryos were exposed to the action of Perenyi's fluid for from ten to twenty minutes; they were then washed and preserved in seventy per cent alcohol. The material from which the best results were obtained remained in the alcohol for three or four months. The eggs were then mounted,

unstained, in a mixture of equal parts of glycerine and water. As the water evaporated it was replaced with pure glycerine. In the course of two or three days, in successful cases, the eggs became so beautifully transparent as to show every detail of structure with the most perfect clearness. In such preparations every nucleus, whether resting or in any of the stages of mitosis, stood out conspicuously. It was thus possible to follow all the divisions of the cells; and I can state that I have seen every spindle, up to a stage containing over fifty cells, more than once, and most of them many times.

Another method that may prove useful to others, and which I have used with success, is to kill in Perenyi's fluid and preserve the material in fifty per cent glycerine. Schneider's acetic carmine may be used for staining in this case.

For later stages Merkel's fluid is a splendid reagent. Corrosive sublimate also gave excellent results. For staining, Grenacher's borax carmine and Mayer's haemalum were used chiefly. Good whole mounts in glycerine of young larvae were obtained after the use of one tenth per cent, or even five hundredths per cent, osmic acid. To kill the glochidia with the shells open, I first added chloral hydrate to the water containing them, and, in due time, any desired killing reagent.

In all the earlier stages sections of the eggs were made *en masse*, and no difficulty was experienced in orienting sections thus made. It is a simple matter to orient the glochidium before sectioning. In hard paraffine (58° C.) the shell of the glochidium is no obstacle to sectioning.

(c) *Nomenclature.*

One of the practical questions which presents itself to the student of cell-lineage is the system of nomenclature to be adopted for the individual blastomeres. The requirements are: (1) a separate designation for each cell which will indicate its approximate location and exact ancestry in the plainest possible way, and (2) the system must be capable of indefinite expansion. These requirements sound difficult, and the fact that so far no two workers have adopted the same

system proves the difficulty to be real. Yet, if the best results are to be obtained from the comparison of the cell-lineages of different animals, some fairly uniform system of nomenclature must be adopted by the different workers in the field. For the sake of uniformity I have adopted the system followed by E. B. Wilson in his "Cell-Lineage of Nereis."¹

The first four cells are designated by the capital letters *A*, *B*, *C*, and *D*; the generations of ectomeres by the small letters *a*, *b*, *c*, *d*; the *first* index number indicates the generation to which the ectomeres belong: thus, a^1 or $b^{1.2}$ or $c^{1.1.1}$ all belong to the first generation, c^2 , $d^{2.1}$, $a^{2.2}$ belong to the second generation, and so forth. *A*, *B*, *C*, and *D* and a^1 , b^1 , c^1 , and d^1 are retained throughout for the four vegetative and four apical pole cells, respectively. When a cell divides, the products receive the designation of the parent cell with the addition of a further index number: thus, $a^2 \begin{smallmatrix} a^{2.1} \\ a^{2.2} \end{smallmatrix}$; the larger index 2 is used for the cell lying nearer the vegetative pole. Exceptions to this rule are made only in the cases of important cells, which receive special designations. Thus the first somatoblast is d^2 ; this designation is replaced by *X*, and the small cells formed from it by x^1 , x^2 , etc. The mesoblast is indicated by *M* for the teloblast and *m* for the smaller cells; in the case of the larval mesoblast the designation $a^{2.2}$ is replaced by *Y*. For the rest, the system will develop as the account proceeds.

Another matter which demands a preliminary explanation is the orientation given the figures of segmentation. I have oriented them all with the part, usually placed below, above. The reason for this is that it obviates the confusing changes of orientation which would otherwise be necessary. The chief part of the large (posterior) cell gives rise to the shell gland, which is dorsal; in keeping it above throughout I have had in mind the final orientation of the embryo. This is (for the same reason) the orientation which has been used by other writers on the Unionidae. At the close of the work there is inserted a section on the change of axes.

¹ The system of nomenclature lately proposed by Mr. Kofoed (No. 49 a) is too rigidly symmetrical to be applicable to a cleavage so irregular as that of *Unio*.

(d) *Cleavage.*

I. THE FIRST TWO FURROWS.

The unsegmented ovum adheres to the vitelline membrane only in the region of the micropyle. The polar globules are invariably formed just opposite to this point. This fact has been commented upon by other authors and I have satisfied myself with merely observing it. The significance of the fact seems, however, to have escaped notice. The micropyle marks the point of detachment from and, in earlier stages, the point of attachment to, the ovarian wall (*cf.* Stauffacher, No. 59). We can thus trace back the orientation of the ovum to the earliest stages of its development in the ovary. The polar globules cannot form at any point, but must form at *one* point in this almost alecithal ovum. The animal and vegetative poles, and therefore the relations of the ectoderm and entoderm are (normally) determined *before the ovum leaves the ovary*. The importance of this fact in the interpretation of cleavage is sufficiently obvious.

The first segmentation plane divides the ovum into two unequal portions *AB* and *CD* (Pl. I, Fig. 4). The division runs from the animal to the vegetative poles and is inclined at an angle of 45° to the future longitudinal and transverse axes of the embryo. (Text — Fig. 1.) When first fully formed the two cells are round and meet over a comparatively small area (Fig. 4); but they at once begin to press against each other and to flatten at the point of contact (Fig. 5). This process may go on until the whole egg has assumed again nearly the form of a sphere; the only external indication of the separation of the two parts being a shallow constriction where they meet. But no actual fusion of the cells ever takes place, for in section there is always a sharp line of division. The smaller cell *AB* is clearer than the larger cell *CD*, as both Rabl and Flemming have remarked; but this difference in appearance is to be ascribed rather to its smaller size and hence greater transparency, than to any marked histological differentiation.

Each cell contains entoderm as well as ectoderm, hence Rabl's designation of animal cell (*AB*) and vegetative cell (*CD*) is inapplicable (No. 25).

The second plane of division is likewise meridional, and practically at right angles to the first. The two cells divide at different times, and these two divisions taken together represent what is usually called the second furrow in other eggs. In the two-cell stage then there arises a certain independence in the times of cleavage of the blastomeres. Later when one cell gains a start, so to speak, over the corresponding cell of another quadrant, it continues to maintain or may even increase its lead. This difference in the time relations of the divisions of the cells of different quadrants is one of the most striking features in the cleavage of *Unio*. The four cells of a quadrant never divide synchronously; the difference in time may be slight but it remains constant throughout many divisions.

CD is the first to divide; the products of division are unequal (Pl. I, *C* and *D*, Figs. 6 to 9). The smaller cell *C* lies on the right side of the future embryo;

D is much larger and occupies the posterior end of the embryo. During the division of *CD*, *AB* begins to constrict (Pl. I, Fig. 6), and shortly after the separation of *C* and *D* divides into two approximately equal parts (Pl. I, Figs. 8 and 9). Of the four cells now making up the embryo *A*, *B*, and *C* are approximately equal; *B* is as a rule slightly smaller than *A* or *C*; *D* is very much larger than any of the other three. (Text — Fig. 1.)

This early division of *CD* is the first indication of a tendency to progressively more rapid cell multiplication in the posterior

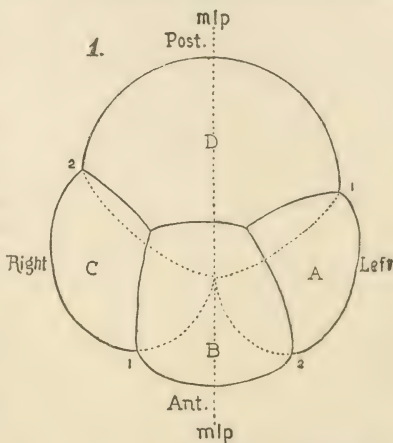


FIG. 1. — Four-cell stage. mlp = median longitudinal plane of larva
1 — 1 = First cleavage-plane.
2 — 2 = Second cleavage-plane.

part of the embryo, which leads at last to fundamental disturbances of the axial relationships of the parts.

Figs. 9 and 10 (Pl. I) show two different phases of the four-cell stage. In the earlier phase (Fig. 9) *B* and *D* meet over a small area at the apical pole, the usual Brechungslinie or cross-furrow of authors. In the later phase (Fig. 10) *B* and *D* meet over a wide area. There is now an extensive cross-furrow at the animal pole. *A* and *C* meet over a very small area at the vegetative pole. In most forms, *e.g.*, Clepsine, Nereis, Crepidula, if either cross-furrow be the larger, it is that at the vegetative pole. The difference finds its explanation in the fact, that the greater mass of the first four blastomeres is ectodermal in *Unio* and entodermal in the above mentioned forms. The special character of the cross-furrow is hence due to the accumulation of the specific plasms at their own poles of the egg. E. B. Wilson comes to practically the same conclusion when he says that the reduction of the apical cross-furrow as compared with that at the vegetative pole in mollusks and annelids "stands in obvious relation to the different size of the cells produced at the two poles."

The orientation of the embryo is now a matter of no difficulty. The large posterior cell *D* maintains its much larger size in relation to the other cells of the embryo. Its more numerous divisions, moreover, give a most characteristic and unmistakable appearance to the posterior end of the egg.

Previous authors have failed completely to understand the significance of these first four blastomeres. Rabl held that *A*, *B*, and *C* were ectomeres, and that *D* formed all of the entoderm and mesoderm as well as some ectoderm. Flemming expressed no opinion on the subject, and Schierholz followed Rabl. As a matter of fact, each of the four cells contains both ectoderm and entoderm. The mesoderm is formed later from *D*, but in a totally different way from what Rabl thought, as will be shown later. In addition to ectoderm and entoderm *A* forms a special kind of mesoblast, for which I shall retain the term larval mesoblast, which I used in my preliminary paper.

2. THE FIRST GENERATION OF ECTOMERES.

Shortly after the division of AB a small cell d^1 is budded off from D towards the apical pole. We have thus a five-cell stage which has been figured by Rabl and others. The three other cells, A , B , C , next bud forth small cells a^1 , b^1 , and c^1 likewise towards the apical pole. These three divisions do not take place synchronously, but in the almost invariable order C , A , and B , so that six, seven, and eight-cell stages occur. The four apical cells thus formed are the first generation of ectomeres.

Fig. 13 is a view of the apical pole in the six-cell stage. D and C have already divided, and it will be seen that both A and B are preparing to divide. In both of these cells the aster to the left belongs to the ectomere; the one to the right is sunk deep in the substance of the cell, and hence is represented as being fainter. This figure shows further that the oblique nature of the cleavage is already indicated by the spindles. Thus the spindle in B points to the space between B and C , that in A to the space between A and B . Fig. 14 from the vegetative pole shows the time relationships of the cleavage very well; c^1 is already separated from C , though the asters are still visible in both cells; the metakinetic stage of division has been reached in A , while B is in the equatorial-plate stage. Fig. 15 is a view from the apical pole of the completed eight-cell stage. It will be seen at once that d^1 is the largest of the ectomeres. Fig. 16 is a view of the same stage from the right side, and partly from the vegetative pole.

Fig. 12 illustrates an interesting condition which occurs but rarely. The four macromeres are of more nearly equal size than usual; in this case A is larger than D . This may, perhaps, be a reversion towards a more primitive type in which the segmentation was equal. It may not be out of place to mention here that all of the eggs obtained from one individual of *Unio complanata* divided equally at the start. As it was impossible to keep the eggs alive long enough, I cannot say that normal embryos would have been produced. But from the vigor with which the early cleavages took place I feel convinced that such would have been the case under normal conditions. Watasé (No. 60) has

also noticed that "eggs from the same animal show similar variations in cleavage"; he concludes that "such a tendency to vary may become hereditary."

The first generation of ectomeres occupy a position in relation to the macromeres which might be supposed to be reached by their rotation after formation through an angle of 45° in the direction of the hands of a watch. This arrangement is obviously well adapted to economize space; if it were produced by an actual rotation of the cells after their formation there would be no difficulty in understanding it. But the fact that the cleavage spindles are oblique, even before the lobing of the cytoplasm, shows that the process is not a purely mechanical one, in the sense that it is produced by the mutual pressure of the blastomeres. This manner of division is characteristic of the ovum of molluscs (cephalopods excepted), annelids and polyclads. Selenka (No. 58a) was one of the first authors to describe it. Speaking of the four ectomeres, Selenka says that they are budded off from the four macromeres "im Sinne einer laetotropen oder λ -Spirale." Blochmann (No. 35), who was the next to describe this type of cleavage, in a proso-branch, *Neritina*, used a different method of expression; he said that, looked at from the animal pole, the first generation of ectomeres during their formation underwent a shifting "im Sinne des Uhrzeigers," and the second generation "in der Uhrzeigerbewegung entgegengesetzten Sinne." Lang (No. 53) used the same form of expression as Selenka; but neither of these authors has made use of the term spiral to distinguish a special type of cleavage. Wilson, on the other hand, has characterized this form of cleavage as the "spiral" type in distinction from his "radial" and "bilateral" types. Heymons (No. 47) seems to prefer Blochmann's comparison with the hands of a watch, for he simply refers to the other expression as "sog. spiralige." Kofoid (No. 49a) uses the term spiral in the same way as Wilson. It seems, then, that there have been two ways of describing this form of cleavage: first, by the use of the term spiral, and second, as a rotation.

Wilson (No. 65, p. 600) derives the "spiral" from the "radial" form of cleavage by "a twisting of the radii, as it were, the

blastomeres being displaced or rotated with respect to the egg-axis, either to the right, following the hands of a watch (right-handed spiral), or in the reverse direction (left-handed spiral), as the case may be." "The term 'spiral' refers to the fact that the curved radii, if prolonged, would form a spiral about the egg-axis." In the ontogeny there is no twisting of the radii, but merely an inclination of the axis of the dividing cell from the vertical. It seems to me, therefore, that this form of cleavage would be more correctly termed *oblique*.

In the radial type cleavages are either vertical or horizontal with respect to the egg-axis; the cleavage spindles are hence, respectively, horizontal or vertical. In the oblique type the second cleavage spindle is not horizontal, but oblique. From the point of view of an observer in the axis of the egg, the spindle is inclined from right below to left above, which can be expressed by the single word *leiotropic*. In the third cleavage the spindle is *dexiotropic*. Regarding the ovum from the animal pole, the upper cell lies to the left of the lower, *i.e.*, in a *leiotropic* position, in the first instance, and to the right, *i.e.*, in a *dexiotropic* position, in the second instance. The second cleavage of the macromeres is *leiotropic*, and the third *dexiotropic* again. In the following pages the cleavages will be described as *leiotropic* or *dexiotropic* according as the inclination of the cleavage spindle from below above is to the left or right of the vertical axis of the ovum, and not according to the direction of the actual planes of division.

Crampton (No. 41a) has discovered that in *Physa*, a sinistral pulmonate, the directions of the cleavages are reversed. Thus, the spindle in passing from the four to the eight-cell stage is *leiotropic*, not *dexiotropic* as in the other cases cited. Inasmuch as the obliquity changes from right to left, or *vice versa*, with each successive cleavage, as in the other cases, the mesoblast is formed from the *right*, not the *left*, posterior macromere. The direction of the division of the left posterior macromere at the fourth cleavage would throw its product on the left side of the embryo, while the homonymous product of the right posterior macromere is thrown in the middle line behind. It would seem in this case that the position of the

fourth product determined whether or not it is to be mesoblast; but that this conclusion is not justified is shown by the fact (which Mr. Crampton very kindly permits me to add) that the macromere which is to form the mesoblast divides before the other. Its prospective function is structurally outlined before this cleavage. For the origin of this reversed mode of cleavage we must go back to the two-cell stage. The cleavage from two to four cells is leiotropic in Limnaea, and dextrotropic in Physa; on this depends the localization of the mesoblast in the first case in the left, in the second in the right posterior macromere. Some structural difference of the two ova conditions the primary difference in the direction of the cleavage, on which all subsequent variations depend.

This is the first time,¹ as already stated (No. 21), that an eight-cell stage has been found to exist in the lamellibranchs resembling the form so characteristic for this stage in all other Mollusca (*Dentalium* excepted), with holoblastic ova, as well as in Annelida and Polyclada, not to mention numerous other forms. This stage, in its typical form, consists of four micromeres lying upon and alternating with four macromeres, the former being derived one from each of the latter, and lying to the right of the parent macromere. It is true that Flemming describes the origin of the eight-cell stage in Anodonta in the same way as I have done, but he does not figure it clearly. He says (p. 130, *l.c.*): "Nachdem der Keim in dem Stadium der Fig. 10" (four-cell stage) "wieder länger mindestens mehrere Stunden geruht hat, beginnt der nächste Act der Theilungsarbeit, und zwar wieder in analogen Weise wie der letzte: auch jetzt theilt sich der dunkle Obertheil (*i.e.*, *D*) in zwei ungleich grosse Segmente, andererseits proliferirt der jetzt dreizellige Untertheil: nur laufen diese Processe hier

¹ Korschelt and Heider state in their "Lehrbuch" that Lankester described for *Pisidium* an eight-cell stage formed of two superimposed layers of four cells each, and they refer the reader to his well-known article (No. 54). Any one who will take the trouble to read the first two pages of his work and to look at his Fig. 17, will find that Lankester described *four* meridional furrows before the appearance of the "first circumferential," or equatorial furrow. Thus the eight-cell stage would consist of eight cells in one plane. This is undoubtedly an error of observation. It is, however, different from the account of Korschelt and Heider.

nicht gleichzeitig nebeneinander ab," *etc.* He then describes in more detail the origin of d^1 (5 of Flemming) exactly as I have done, and goes on to say (p. 131, *l.c.*): "Inzwischen schicken auch Zelle 2, 3 und 4 (*C*, *B* and *A*) zur weiter Theilung an, so aber dass diese erst nach der Produktion von 5 (d^1) und nicht bei allen drei Zellen gleichzeitig erfolgt." I am very glad to find my statement of these simple and easily-observed facts in such exact agreement with Flemming; the more so, as I am forced to differ from Rabl, who describes d^1 as dividing immediately after its formation, a thing in itself but little probable.

I am the more particular in emphasizing these facts, as Korschelt and Heider, in the third part of their "Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere," make the following statements about the cleavage of the lamellibranch ovum: "Die Furchung stimmt bei denjenigen Formen bei welchen sie genau untersucht wurde (*Unio*, *Anodonta Cardium*, *Cyclas*, *Teredo*) in so auffallender Weise überein, dass man auf einen ähnlichen Verlauf derselben auch bei den Formen schliessen darf von denen einzelne ähnliche Stadien bekannt geworden sind (*Ostrea edulis*, *Pecten*, *Mytilus edulis*). . . . Die Furchung ist stets inäqual. . . . Die kleine Furchungskugel theilt sich in zwei und ungefähr gleichzeitig oder wenig später schnürt sich von der grossen eine neue Furchungskugel. Auch diese theilt sich und sodann wiederholt sich derselbe Vorgang der Abschnürung eines Mikromers von der grossen Furchungskugel. Ganz der gleiche Process findet nochmals statt, indem von der grossen Zelle kleinere geliefert werden, welche sich sodann theilen. Die Mikromeren sitzen schliesslich, von der Oberfläche gesehen, wie eine Kappe auf der grossen Furchungskugel, welcher letzere erst später sich in zwei Zellen theilt."

It is hardly necessary for me to state that I cannot accept this as a general scheme applicable to all lamellibranchs. If one looks over the literature on lamellibranch embryology, one is struck by nothing so much as the meagerness of the details on cleavage. It has generally been thought sufficient to describe it as far as the six or seven-cell stage and to say:

"the same thing is repeated and results in the formation of a cap of small cells lying on a large one." The large cell is held to be entodermal always, but in *Unio* I am certain that it is not so.

It seems impossible at present to reduce the two hitherto described forms of cleavage in lamellibranchs to a common law; but Korschelt and Heider compare Rabl's account of the cleavage of *Unio* with Hatschek's account of *Teredo*, in spite of the fact that the first cleavage of the larger cell is meridional in the first case and equatorial in the second, according to the description given. According to Ziegler and Stauffacher, the cleavage of *Cyclas* is like that of *Teredo*; the latter's sections are convincing; one can no longer doubt that the first cleavage of the larger cell is equatorial in this form. How this type of cleavage can be derived from the oblique type, it would be difficult to say. But perhaps the only difference is that the obliquity of the spindle is greater.

3. FROM THE EIGHT TO THE SEVENTEEN-CELL STAGE.

A nine-cell stage is formed in *Unio complanata*. This stage is reached from the eight-cell stage by a division of the posterior macromere *D* in an equatorial plane (Pl. II, Figs. 17 and 18). The division is unequal, the ectomere d^2 being much larger than the macromere¹ *D*. d^2 is the "first somatoblast" (v. Wistinghausen) and in succeeding stages will be designated by the letter *X*. It is also the first of the second generation of ectomeres. The formation of the other members of the second generation of ectomeres is illustrated in Fig. 19, Pl. II (a view from the right side); a^2 , b^2 , and c^2 are larger than their parent macromeres *A*, *B*, *C* (Pl. II, Figs. 20 and 21). The cleavage-spindles of the second generation of ectomeres are leiotropic.

d^1 divides next, thus producing the thirteen-cell stage (Pl. II, Fig. 20, apical pole). The divisions of the other members of the first generation of ectomeres soon follow. The order of

¹ The term "macromere" will be retained throughout for the four hypotrophic cells (Goette) without reference to their relative size.

the division is c^1 , a^1 , and b^1 , thus repeating the order of the first divisions of D , C , A , and B .¹ Figs. 22 and 23 (Pl. II) illustrate the appearance of the apical and vegetative poles respectively in the seventeen-cell stage. The seventeenth cell is x^1 (Fig. 23), which has been budded forth from X just behind C on the vegetative pole. (Cf. Figs. 20 and 24 of *Nereis*; Wilson *l.c.*) The first division of the first generation of ectomeres is leiotropic.

4. FROM THE SEVENTEEN TO THE THIRTY-EIGHT-CELL STAGE: THIRD GENERATION OF ECTOMERES, ETC.

The eighteen-cell stage is reached by another division of D (Pl. II, Fig. 23). The cell to the left is the first member of the third generation of ectomeres (d^3 , Figs. 25 and 27). A period of rest now ensues. When activity is again resumed, spindles appear almost simultaneously in a^2 , b^2 , c^2 , and X (d^2). The positions of these spindles and, in consequence, of the resulting cells is very different in the different cases. Figs. 24, 25, and 27 (Pl. II) illustrate the description. A cell, x^2 , is budded off from X on the left side, symmetrical with x^1 on the right side and just posterior to d^3 (Pl. III, x^2 , Fig. 29). The cleavage of c^2 and b^2 is equal and dextrotropic. The division of a^2 calls for special attention, inasmuch as the larval mesoblast is separated by it. Fig. 25 shows that the division will be equatorial. After the completion of the division it is seen that the cell nearer the vegetative pole (Pl. III, $a^{2.2}$ or Y , Fig. 29), which becomes the larval mesoblast, is the larger. Y is bounded by the following cells: on the right by x^2 and d^3 , on the left by $d^{1.1}$ and $a^{2.1}$, behind by X , and in front by A and part of D . Already (Pl. III, Fig. 29) it is partially covered by x^2 and d^3 .

During these divisions the cells of the apical pole lose their rounded contour and together form a flat plate of cells (Pl. II, Fig. 26). They now enter upon a long period of rest, during which extremely important changes, which lead to the establishment of the mesoblast and entomeres, take place at the

¹ Lang has observed that in *Discocoelis* the first generation of micromeres follow the same rhythm of division as their parent macromeres (No. 53, p. 331).

vegetative pole. Before passing on to a description of these changes, I will direct attention to another division of X (Pl. III, Figs. 31 and 33), by which a small cell is budded off towards the apical pole just posterior to $d^{1.2}$ (Pl. III, x^3 , Fig. 35). The appearance of this cell at this time and place is extremely interesting, seeing that it tallies exactly with Nereis (E. B. Wilson *l.c.*, Pl. XVI, Fig. 33).

The remaining members, a^3 , b^3 , and c^3 , of the third group of ectomeres are now formed by the simultaneous leiotropic division of A , B , and C (Pl. III, Figs. 32 and 34). After these divisions the entoderm is definitely localized in the four cells A , B , C , and D (Pl. III, Fig. 38). The larger part of D is, however, mesoderm, which in the next figure (39) is shown about to be definitely separated. *When this division is completed (Pl. IV, Figs. 41 and 42) the delimitation of the germinal layers in distinct blastomeres is accomplished.* The other spindles in Fig. 39 explain themselves, and the resulting cells are shown in Fig. 42.

The embryo at the time of the separation of the germ-layers contains thirty-two cells (see table of cleavages, p. 33). At the same time the embryo of Nereis contains thirty-eight cells. The difference is due to the suppression of cleavage in the apical pole cells of Unio. The composition of the embryo can be gathered from the following table:

| | |
|--|----------|
| Entomeres. $A-D$ | 4 |
| Mesoblast. $M (d^4)$ | 1 |
| Larval Mesoblast. Y and y^1 | 2 |
| Ectomeres of first generation | 10 |
| Ectomeres of second generation ($b^{2.1}$, $b^{2.2}$, $c^{2.1}$, $c^{2.2}$ and $a^{2.1}$) | 5 |
| First Somatoblast | 6 |
| Ectomeres of third generation | 4 |
| | <hr/> 32 |

The number (32) of cells at this stage is a chance coincidence merely; it has not been reached by a geometrical progression from two, four, eight, sixteen, to thirty-two cells, as in a synchronously cleaving ovum.

Almost every cell in this stage has so distinctive a character that if isolated from the cell-complex it could be recognized

by its general form. The embryo has the appearance of an irregular mosaic.

After the establishment of the germ-layers in separate cells, and before the beginning of bilateral divisions, a fourth division of the first somatoblast takes place. A small cell (x^1 , Fig. 39) is budded off anteriorly towards the vegetative pole and against the posterior end of the second somatoblast. This division of X does not occur in this form in *Nereis*. The fourth division in *Nereis* is equal and bilateral, whereas in *Unio* the fifth is the first bilateral division. Other divisions take place at about this time on or in the region of the vegetative pole, which give it a most characteristic appearance. These are the divisions of x^1 , d^3 and Y . x^1 buds off a small cell towards the antero-lateral border of the second somatoblast ($x^{1.1}$ and x^1 Figs. 39 and 40). A division of exactly the same general form takes place in *Nereis* (No. 64, Figs. 52 to 54). d^3 divides somewhat later ($d^{3.1}$ and $d^{3.2}$, Figs. 40, 42, and 45; $d^{3.2}$ helps in the overgrowth of M). This division of d^3 is interesting from the fact that the other micromeres of the third generation do not divide till much later. d^3 has apparently inherited the tendency of its parent macromere D to rapid division. Y buds off y^1 , between d^3 , A and a^3 (Pl. IV, Figs. 39 and 40), and a little later y^2 , on the other side (Pl. V, Fig. 59).

While dealing with these divisions it will be just as well to include the observations which I have made on the further divisions of the first and second groups of micromeres. If the order of division were the order of description it would be necessary to postpone this for some time later, but in that case, I fear that the reader would be as tangled up in the description as I was at one time in the apparently confused and indeterminate order of the facts. I have followed the first group of ectomeres to a stage when sixteen cells of this group are formed. It would have required much time and trouble to have followed them farther: and, inasmuch as no larval apical organs are formed, I desisted. I have not, therefore, found the cross on which Conklin lays so much stress in *Crepidula*; I doubt very much its existence in any stage in *Unio*, for I should certainly have seen it, were it formed.

I have no doubt that there is a causal relation between the rudimentary condition of the pre-velar region and the slow and irregular character of the cleavages of the first generation of ectomeres which forms this region.

d^1 is generally the first of the apical pole cells to divide. The products are d^1 and $d^{1,2}$, which abuts against x^3 . c^1 generally follows, and $d^{1,1}$ about the same time. Then comes $c^{1,1}$ and a^1 , followed by $a^{1,1}$; b^1 and $b^{1,1}$ are the last of the ectomeres of the first generation to divide. After their division a spindle appears in $d^{1,2}$. Fig. 51 exhibits an unusually regular arrangement of these sixteen apical cells. It can easily be seen there that, while the first division of the four central cells a^1 , b^1 , c^1 , and d^1 was leiotropic, the second division is dextiotropic.

We have already noticed one division of the three anterior members of the second generation of ectomeres. This division was obliquely equatorial in the cases of b^2 and c^2 , and much more nearly horizontal in the case of a^2 . In this latter case the variation is correlated with the formation of the larval mesoblast. We shall treat the divisions of d^2 (the first somatoblast) and $a^{2,2}$ (the larval mesoblast) separately, and so have now to concern ourselves simply with $b^{2,1}$, $b^{2,2}$, $c^{2,1}$, $c^{2,2}$, and $a^{2,1}$. $b^{2,2}$ and $c^{2,2}$ lie nearer the lower pole than $b^{2,1}$ and $c^{2,1}$; $b^{2,1}$ and $b^{2,2}$ are of approximately equal size; the same is true of $c^{2,1}$ and $c^{2,2}$. These four cells divide almost simultaneously, though $c^{2,1}$ leads the other three by a little. The plane is in each case nearly horizontal (Figs. 41, 42, 43, 45, and 47); each cell divides somewhat unequally: in the cases of $b^{2,2}$ and $c^{2,2}$ the smaller product lies nearer the vegetative pole. The reverse happens with $b^{2,1}$ and $c^{2,1}$; $b^{2,2,2}$ comes to lie in the angle enclosed by a^3 , A , B , and b^3 . This group of cells has a very characteristic appearance, as shown in the figures (*e.g.*, Figs. 42 and 43). Fig. 43 is an anterior view of the egg, and shows very clearly the divisions of $b^{2,1}$, $b^{2,2}$, and $c^{2,2}$. The spindle of division of $c^{2,1,2}$, which I have several times seen, is shown in Figs. 45 and 47. As for $a^{2,1}$, a single division of this cell is represented in Figs. 48 and 49, and the two resulting cells are shown in Figs. 50 and 51.

What is the fate of the second generation of ectomeres? The answer will be given separately for d^2 and $a^{2,2}$; the others form the larval mantle, or, at least, contribute to its formation. This being so, we have a satisfactory explanation of their large size, which is due to the precocious segregation of this large and important organ in single cells. The text figures on p. 59 show the relation of these blastomeres to the future embryonic areas.

5. ESTABLISHMENT OF BILATERAL SYMMETRY. THE LARVAL MESOBLAST.

The spindle of bilateral division of the first somatoblast is seen in Fig. 44 (Pl. IV). This is a view from behind of a stage slightly older than Fig. 42 (Pl. IV). Shortly after the completion of the division indicated, each of the resulting cells buds forth a small cell x^5 towards the vegetative pole (Pl. IV, Figs. 45, 46, and 47). These two small cells are placed just behind $x^{1,1}$ and x^4 (Pl. IV, Fig. 45) and form with the cells x^1 , $x^{1,1}$, x^2 , and x^4 the beginning of a tongue of cells, which grows forward and over the second somatoblast. Between the entomeres and the two cells X, X (the protoblasts of the shell-gland) there are no cells after the inclusion of the mesoblasts but the derivatives (x^1-x^5) of the first somatoblast. Therefore there is no room for doubt, that, after the invagination of the entomeres and inclusion of the mesoblasts, all of the cells lying between the blastopore and the posterior end of the shell-gland are derivatives of the first somatoblast. I anticipate here what will be shown in detail later, *viz.*: that these cells form the ciliated plate (Wimperschild of Flemming) from which the foot is derived later on.

The next bilateral division is that of the second somatoblast (mesoderm protoblast) M (Pl. IV, Fig. 45). Fig. 46 shows the two mesoblasts just after the completion of this division. The invagination of the entoderm is slightly indicated in this figure, and its position is sufficient proof that the cells concerned are A, B, C , and D .

With these two divisions (*i.e.*, of the first and second somatoblasts) the bilateral character of the cleavage becomes apparent. By this I do not mean to say that there is a complete cessation

of oblique divisions ; on the contrary the micromeres of the first generation continue for some time to divide obliquely and the same holds true for other cells. But from now on there is no difficulty in recognizing the fact that we are dealing with a bilateral embryo, and little by little all parts of the embryo are brought into relations of bilateral symmetry.

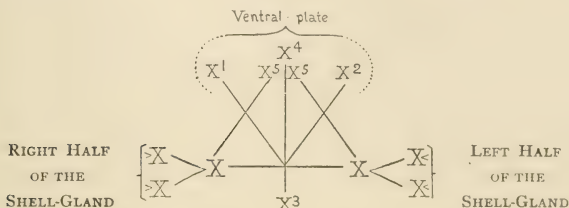
There is no more striking instance of this than in the behavior of the larval mesoblast Y , which during these changes has been more and more overgrown by the surrounding cells (Pl. V, Fig. 59). It has budded off two small cells y^1 and y^2 (Pls. III, Fig. 39 and V, Fig. 59), and I am inclined to think, though I am not certain of it, that a third small cell is budded from Y , before the latter enters the segmentation cavity. Fig. 61 is an optical frontal section of a stage with four large dorsal cells, products of X ; the larval mesoblast is here seen to lie almost entirely within the segmentation cavity. Fig. 62, an actual transverse section of a still later stage, shows Y in the process of equal bilateral division. *Thus, though in origin asymmetrical, the larval mesoblast comes, apparently by active migration, to be placed symmetrically in later stages.* In Fig. 62 the next important change in the history of the larval mesoblast is shown. Each half is dividing. The resulting cells are shown in section in Fig. 66 (Pl. V). In some of my preparations I have seen spindles in the two cells of the larval mesoblast, which would lead to the formation of extremely small cells lying against the ventral wall of the embryo near the region where the oral plate later appears. In yet others I have seen these cells fully formed. I am not certain that this always takes place, but when it does it always precedes the division just mentioned.

6. THE FIRST SOMATOBlast.

To return to the cells X , X , from which the shell-gland is formed. Fig. 60 shows the right cell already divided into fairly equal parts and the left in process of division. The order of division of these two cells is invariably as figured ; the two divisions are never synchronous, but I have not been able to correlate this difference of time relations with any

future difference of the two sides of the shell-gland. The four resulting cells are seen faintly outlined in Fig. 61, which also shows that the two anterior cells are somewhat the smaller. By repeated divisions a plate of high columnar cells is formed (Pl. V, Figs. 64, 66, and 72), which occupies the whole of the future dorsal region of the larva. It has caused an enormous expansion of the area previously occupied by the cell *X* (cf. Pl. V, Figs. 59 and 72), and has thus completely altered the embryonic topography (*vide* text figures, p. 59), *establishing the permanent dorsal axis of the larva and adult.*

Let us tabulate the divisions of the first somatoblast :



The cells referred to the center of the bar joining the two central *X*'s, are those which were budded from the first somatoblast before its bilateral cleavage. The table indicates that the first somatoblast is the protoblast of two very important organs, *viz.* : the shell-gland and the ventral plate.¹

I cannot forbear calling attention again to the wonderful similarity of the cleavages of the first somatoblast in *Nereis*. (Cf. table on p. 410 of E. B. Wilson's *Nereis* work.) In *Unio* the first somatoblast buds forth four small cells, whereas in *Nereis* it buds forth three small cells only, before its bilateral cleavage ; the place of formation and relative size of the cells is exactly the same as in *Unio*. One would expect from its original position that it would form the mid-dorsal region in *Nereis* as in *Unio* ; and as a matter of fact such seems to be

¹ I use the term "ventral plate" with full knowledge of its significance in annelid embryology. The term is, however, so applicable to this tongue of cells, and the fate of the cells in question is so similar in part to those of the Annelid ventral plate that I feel justified in using the term.

the case. However, according to Wilson, "*the residual teloblasts move apart so as to leave a triangular space between them covered with small transparent cells (dor.) and at the same time they gradually recede from the prototroch towards the lower pole.*" This change in position of the teloblasts is of fundamental importance, since I believe the triangular area to represent the middle dorsal region of the adult body, and the residual teloblasts to mark the posterior limit of the ventral plate." (The italics are Wilson's.) This quotation alone would give one the impression that the middle dorsal region was not formed from the first somatoblast. However, later on, p. 419, Wilson says that "the small cells which separate the posterior teloblasts from the prototroch" are "the descendants of x^3 and (?) of x^6 , x^6 . From the latter, as I believe, arise the cells that occupy the triangular area between the two residual teloblasts after their divergence. This area afterwards forms the middle dorsal area of the trunk." Thus in *Nereis* as in *Unio* the median dorsal as well as the ventral surface is a product of the first somatoblast. There is in *Nereis*, but not in *Unio*, a latero-dorsal strip of the trunk epiblast which is derived from a^2 and c^2 . In *Nereis* the formation of the ventral surface from the first somatoblast is unmistakable, the origin of the dorsal surface is not so apparent. In *Unio* the reverse is the case; thus at first sight it seems as though there were a reversal of surfaces, so that cytogenetically the dorsal surface in *Unio* would correspond to the ventral surface in *Nereis*; but that such is not really the case is sufficiently apparent on a closer analysis.

I am under obligations to my friend, Mr. A. D. Mead, for permission to record his observations on this subject. Mr. Mead has found in *Amphitrite*, one of the tubicolous polychaeta, that the collective ectoderm of the trunk, dorsal and lateral as well as ventral, is formed from the first somatoblast. "The small transparent cells" ("dorsal cells" of Wilson) form the dorsal ectoderm of the head segment only. Unless *Nereis* differs radically from *Amphitrite*, Wilson has been in error in regard not to the origin but to the fate of the dorsal cells.

7. THE SECOND SOMATOBLAST.

We have already become acquainted with the lineage of this cell; in the last section we saw further that its first division was equal and bilateral. It will now be in place to trace the further history of the two resulting cells up to the time of their inclusion within the segmentation cavity as the mesoderm teloblasts. Their position is immediately behind the entomeres (Pl. IV, Fig. 46). In this stage the products of the first somatoblast are beginning to overgrow them.

The next division of the mesomeres (Pl. V, Fig. 60) is peculiar. It is very unequal, and the two small cells *m, m*, are budded forth just on the posterior lip of the blastopore. This tallies exactly with *Nereis*. In *Nereis* these superficial divisions are continued for some time, but in *Unio* such is not the case. Soon afterwards the mesomeres are included within the segmentation cavity, and take up their definitive position behind the archenteron (Pl. V, Figs. 66 and 67). v. Wistinghausen has observed that in *Nereis dumerilii* about one half of the second somatoblast remains within the bounds of the ectoderm as the "untere Urzellen des Rumpfes," and forms the anterior part of the ventral plate.

According to Stauffacher's recent observations (No. 59) on *Cyclas cornea*, two cells placed symmetrically on either side of the middle line divide in such a way that about one half of each cell comes to lie in the segmentation cavity. According to Stauffacher the two cells in the segmentation cavity are the protoblasts of the mesoderm; the two cells on the surface the protoblasts of the entoderm. It is to be observed, however, that the last point is a pure assumption. The cells in question cannot be, or, at least, have not been, traced into the entoderm; and there seems to be a total lack of points of orientation for fixing on the region of these cells, and of the later entoderm cells as identical. I am inclined to think that the surface cells correspond to the cells budded off on the surface by the mesoblast in *Nereis* and *Unio*, and that the entomeres lie in front of this point in the form of a plate of relatively small cells. This view, at any rate, accords more

nearly with what we know of mesoderm formation elsewhere; whereas Stauffacher's account stands alone.

Wilson describes the cells which have been budded off at the surface by the primary mesoblast as forming a pigment area, and later wandering within the segmentation cavity as "secondary mesoblast." The significance of this fact in the interpretation of mesoblast cells of "ectodermal origin" can hardly be overestimated. I believe that the same ultimate fate awaits the two superficial mesoblast cells in *Unio*. In Fig. 67 it will be seen that between the most posterior cell of the primitive intestine and the mesoderm teloblast there lies a small cell, which is at the most anterior end of the ventral plate, the exact position of the cells *m, m*. It may be that they are actually these cells, but it is impossible to prove it. In their present position it would be easy for them to be pushed within the segmentation cavity.

After the teloblasts of the mesoderm have entered the segmentation cavity, each buds forth anteriorly a small cell (Pl. V, Figs. 63, 66, and 67). This is the beginning of a mesoblastic germ band on each side. The two bands are parallel, and grow forward in contact in the median line just beneath the large cells of the shell-gland. The teloblasts are forced within the segmentation cavity by the forward growth of the tongue of cells derived from the first somatoblast. The progress of their inclusion can be traced through a series of figures (45, 46, *etc.*). x^{1-3} , x^4 , and x^5-x^5 are very active factors in the process.

(e) *Gastrulation and Shell-Gland.*

The archenteron is derived from the entomeres *A, B, C*, and *D*. Before they invaginate to form the primitive intestine they increase considerably in number (Pl. V, Fig. 60). Even before this stage is reached a slight indentation is noticeable in the region of the entomeres. The invagination deepens, and soon forms a small sac communicating freely with the exterior (Figs. 65, 66, and 67). Fig. 65 is a view of this stage from the ventral surface (the entoderm is colored in sepia). Fig. 64 is a view from the dorsal surface, and Fig. 72, from

the side. These views are given in order to make the external topography of this important and hitherto misunderstood stage clear. In Fig. 72 the reference line running to *Y* passes through the blastopore region. The position of the larval and primary mesoblast¹ are other points of orientation which make comparison with Figs. 64 and 65 easy.

Figs. 66 and 67 (Pl. V) are two successive sagittal sections through an embryo of this stage. Fig. 67 is a true median section, while 66 passes a little to one side of the middle line. The dimensions of the entodermic sac are comparatively insignificant, as one would expect from the slight initial size of the entomeres. In this stage the blastopore has a considerable antero-posterior extent (Fig. 67).

It will be noticed that the primary mesoblast lies behind, and the larval mesoblast in front of the primitive intestine. There is thus no possible chance of confusing the two structures. They are as distinct in appearance and position as in origin. It was indeed this stage which first convinced me of the twofold origin of the mesoblast in *Unio*. By following the clue back I arrived at the results already given.

Previous observers have completely overlooked this stage, or, at least, its anatomy. Goette is the only one who has given a correct account (which is, however, very incomplete) of the origin of the entoderm in the Unionidae, his observations having been made on Anodonta. While he is no doubt right in holding that the invagination of the entoderm occurs at the spot indicated; still my observations both on whole and sectioned larvae of *Unio* lead me to think that an invagination would be found in an earlier stage of Anodonta at the region in question.

If I take up the shell-gland now, it is because of its having been more than once (Rabl, Schierholz) confused with the primitive intestine; also because I have been able to ascertain its cytogeny with certainty, which gives it a place in the first, or cytogenetic division of the paper. The cytogeny of this organ has been already described, so that we can begin

¹ I use the term primary mesoblast for all mesoblast derived from the teloblasts *MM*.

here with the stage of Figs. 64, 66, 67, and 72 (Pl. V), where the gland is represented by a plate of large cells occupying the whole dorsal¹ region. These cells invaginate, and so give rise to the shell-gland, the long axis of which is transverse to the long axis of the embryo (Pl. V, Figs. 69 and 70); it is of an enormous size as compared with other molluscan embryos. This is, of course, a special provision for the needs of the glochidium, which possesses an enormous shell in proportion to its bulk. So large is this gland that its invagination makes an appreciable difference in the diameter of the embryo, as may be seen by a comparison of Figs. 66 and 69, both of which are camera drawings with the same lenses. It might be said that Fig. 69 was drawn from a smaller embryo; but, as a matter of fact, embryos of this stage are always smaller than in the stage just before the invagination of the shell-gland. A large shell-gland seems to be characteristic of lamellibranch embryos. Reference to figures and comparison with the embryos of other Mollusca will illustrate my point.

It is not my intention to dwell on the view which interpreted this gland as the archenteron. That is not, I suppose, any longer held by any one. But it is rather remarkable that two observers, Rabl and Schierholz, should have seen the gland migrate bodily to the region in front of the ventral plate. I can only suggest that the observations were made on partially macerated embryos, in which I have myself seen appearances which might deceive in some such way.

(f) *Summary.*

The first cleavage is unequal; the second divides the smaller cell equally and the larger unequally. The four-cell stage is composed of three subequal and smaller cells and one large cell, which lies at the posterior end. One of the smaller cells is anterior and the other two right and left, respectively. The ectoderm is separated from these four cells in a series of three

¹ The term dorsal here refers to the adult, and not the embryonic axis; the question of axial relations is entered into further on.

oblique cleavages, the first of which is dextrotropic, the second leiotropic, and the third dextrotropic again. The next division (*i.e.*, the fourth) of the posterior macromere separates the protoblasts of the mesoderm.

The first generation of ectomeres divides very slowly. Its cells are destined for the anterior end of the future larva.

The second generation of ectomeres is remarkable for being composed of the largest cells in the embryo. The posterior member d^2 or X is the protoblast of the shell-gland and ventral plate. The larger part of the left member (*i.e.*, $a^{2.2}$ or Y) forms that portion of the mesoblast which I have called the larval mesoblast. $a^{2.1}$, b^2 , and c^2 enter into the formation of the larval mantle. The third generation of ectomeres probably does the same.

The entomeres are small. They undergo division before invagination. The resulting archenteron is extremely small, *but it invaginates before the shell-gland*; thus, though rudimentary, its formation is not delayed.

The shell-gland is a voluminous structure formed from a plate of large columnar cells, which are derived from repeated divisions of X .

The first bilateral cleavage is that of the first somatoblast; the second, that of the second somatoblast.

The primary mesoblasts enter the segmentation cavity and lie just behind the archenteron, thus in the angle which the latter makes with the shell-gland. Their divisions are typically teloblastic. It should be said that, before they enter the segmentation cavity, each buds off a small cell at the surface. The larval mesoblast conforms to the bilaterality of the embryo after entering the segmentation cavity. Its divisions are not teloblastic.

The table of cleavages which follows gives, in some detail, the order of the divisions and the destiny of the blastomeres. The stages indicated by columns separated by continuous lines represent, more or less nearly, natural periods of rest of the entire ovum. The vertical columns united by dotted lines indicate more or less synchronous divisions. This method was adopted to show clearly the very irregular course of the

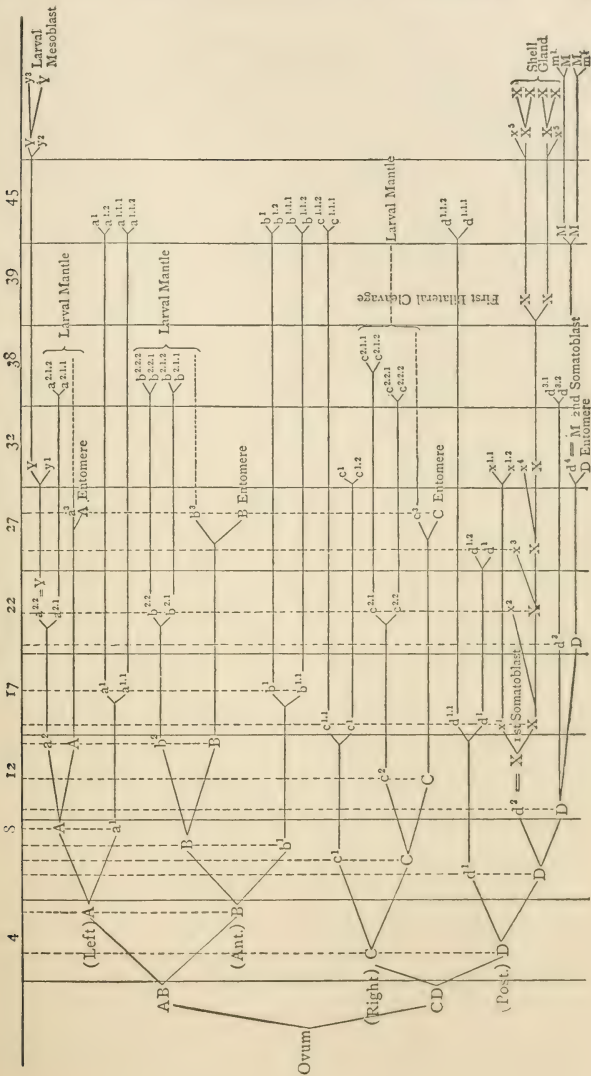
cleavages. For a general survey the dotted vertical lines can be left out of account, and the cleavages within one column thought of as synchronous. I include in an appendix the results of some of the most important works on cytogeny reduced to tabular form. To make comparisons more simple all systems of nomenclature have been reduced to the one employed here; but the original system has, in most cases, been included in brackets, after the common system.

(g) *General Remarks on the Cleavage and Germ-layers.*

Increasing accuracy and detail in the study of the cleavage of the ovum has been one of the most marked tendencies of recent years in embryology. Primarily undertaken, in the Invertebrata at least, to explain conflicting statements as to the origin of the germinal layers in general and the mesoblast in particular, the study of the cleavage is leading to new ideas on the promorphological state of the ovum, and the real nature of differentiation. In the Polyclada, Annelida, and Mollusca, not only the germinal layers, but systems of organs, and even single organs have been traced back to their parent blastomeres, and it has been shown that cells of the same lineage, even in widely separated species, undergo, as a rule, the same ultimate differentiation.

The mesoblast was the first of the germ-layers to be traced back to a single cell. Kowalevsky (No. 52) in 1871 showed that the mesoblast of *Lumbricus* could be traced back to two posterior pole cells, derived from the entoderm, which budded off anteriorly, a large number of small cells thus forming a mesoblastic germ band. He postulated a double source for the mesoderm of *Euaxes*: the larger part came from two large cells derived from the posterior macromere; the rest was derived from two small cells, derivatives of the two lateral macromeres. Rabl (No. 25) formulated his results in 1876 as follows: "Das mittlere Keimblatt entsteht also nach unseren Auseinandersetzungen aus zwei, am Mundrande der Gastrula gelegenen Zellen, deren Verwandschaft zu den Zellen des inneren Blattes eine viel innigere ist, als zu jenen des äus-

TABLE OF CLEAVAGES.



seren." The origin of the mesoblast, from two bilaterally symmetrical cells, has often since been described. Professor Whitman (No. 61, 1878) was the first to show that there was a perfectly definite cell history and origin from single cells for other structures of the adult. He demonstrated that, in Clepsine, not only the mesoblast, but the ventral nerve cord and the trunk nephridia could be traced back to single cells; and further, that the cells representing these structures were the product of the posterior macromere of the four-cell stage, which was thus the representative in this stage of the whole trunk. In 1879 Rabl's paper on Planorbis appeared. He showed, for the first time, that the whole ectoderm was formed in a series of three cleavages each, from four basal macromeres. Blochmann (No. 35), 1882, described three generations of ectomeres in Neritina, and derived the mesoblast from the fourth cleavage of the posterior macromere. During the last twelve years a great many papers dealing with the cleavage have appeared. In this section I wish to point out the most obvious results of the later work on the annelids and molluscs, and to show the bearing of my observations on Unio.

As I have already said, Rabl was the first to show for any form that the epiblast is formed in three generations of ectomeres from four basal macromeres. (Fol described these three generations, but believed that the macromeres contributed to the epiblast after that.) Since then the same thing has been shown to be true for Neritina (Blochmann, No. 35), Umbrella, (Heymons, No. 47), Crepidula (Conklin, Nos. 39 and 40), Limax (Kofoid), and Unio among the mollusca; and for Nereis limbata (E. B. Wilson, No. 64), Nereis dumerilii (v. Wistinghausen, No. 66), Polymnia, Spio, and Aricia (E. B. Wilson, No. 64) and Amphitrite (Mead) among the Annelids.¹ v. Wistinghausen

¹ McMurrich (No. 56) thinks that more than three generations of micromeres are formed in Fulgur. He thinks that "probably the amount of yolk present influences the number of spherules formed." It may, of course, be true that more than three generations of micromeres are formed in Fulgur, just as in Polymnia and Aricia (Wilson, No. 64, p. 458). The important point to determine is how many of these generations are ectomeres. From the recent studies it seems to follow that while the number of generations of *micromeres* is variable, the number of generations

described the mesoblast as formed by the third cleavage of the posterior macromere. E. B. Wilson has already pointed out his probable error. In all of the other cases cited the mesoblast is formed by the fourth cleavage of the posterior or left posterior macromere. Heymons (No. 47, p. 271) has already emphasized this fact.

The accompanying diagrams will serve to emphasize the facts already dwelt on and to bring out others. The first diagram illustrates one point of fundamental importance, *vis.*: That the posterior macromere contains all the elements of the trunk in Clepsine Nereis,¹ Amphitrite, and Unio. The first somatoblast (neuro-nephroblast, Whitman) forms the ventral plate and all ectomerall elements of the trunk; while the second somatoblast forms the mesodermal elements of the trunk. (In no other mollusc (Diagram 2) has the ectoderm of the trunk been traced to the posterior ectomere of the second generation. But from an inspection of Heymons' and Conklin's figures I have but little doubt that the shell-gland at least is derived from this blastomere; the cytogeny of the foot is not as yet known in these forms.) It is probable, too, that in all these cases the members of the first generation of ectomeres form the region in front of the prototroch. This has been shown beyond doubt for Nereis (two sp.) Umbrella, Crepidula Unio, and Amphitrite, and made more than merely probable for other forms. In all cases, too, the second and third generations of ectomeres probably take up homologous positions in the body. The macromeres after the separation of the ectomeres and mesoderm (by the same number of cleavages) become entomeres.

of *ectomeres* is invariable (Wilson, No. 64; Heymons, No. 47) in Annelida and Mollusca; all micromeres after the third generation, with the exception of the posterior micromere of the fourth generation (mesoblast), being entodermic. From Brobetzky's description of Nassa one would conclude that more than three generations of micromeres are formed; but until it is shown that all are ectomeres, we cannot accept this as evidence against the views upheld here. Similarly it is not certain as yet just how many generations of ectomeres are formed in Clepsine. However, it has not yet been shown for any annelidan or molluscan ovum of the oblique type of cleavage that more or less than three generations of ectomeres is formed.

¹ In Nereis a dorsolateral strip of trunk epiblast is derived from A and C, according to Wilson.

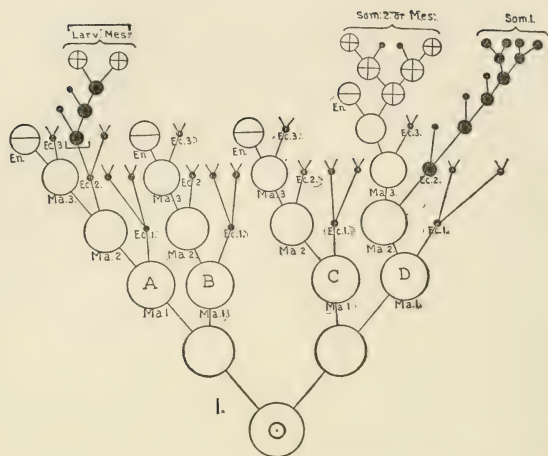


FIG. 1.—Diagram of the cleavage in Clepsine, Nereis, Amphitrite, and Unio. The part in square brackets is peculiar to Unio. In Clepsine the third generation of ectomeres has not been found and the mesoderm is formed from the posterior ma. 3.—ec. 1., ec. 2., ec. 3., first, second, and third generations of ectomeres. ma. 1. to ma. 4., Macromeres of different stages. en., Entomere. Larv. mes., Larval mesoblast. som. 1., First somatoblast. som. 2., Second somatoblast.

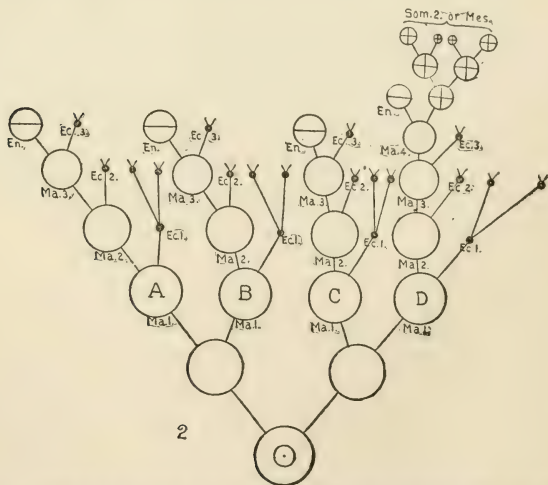


FIG. 2.—Diagram of the cleavage in Crepidula, Umbrella, Neritina, Planorbis, Limax, and Physa.

It is thus possible to speak of an homology of cells. Does it therefore follow that all cells of the same lineage in these different forms are homologous and their products likewise, and that we must deny the homology of cells which, although of different lineage, yet in the end produce homologous organs? I think not. Yet it may be said that if we can speak of homology in the one case, why not in the other? To which it may be answered, that the only safe test of homology *in such cases* is the end result. We have these facts: (1) Cells of the same lineage have the same fate in a wide series of forms (*e.g.*, mesoblasts=fourth cleavage of *D*. (*Cf.* also *supra*.) (2) Cells of the same lineage have a different fate in forms which otherwise agree closely (*e.g.* $a^{2.2}$ in *Nereis* and *Unio*). (3) Cells of different origin have a different fate (numberless instances). (4) Cells of different origin have the same fate, *e.g.*, mesoblast in polyclads and annelids (*cf.* tables 5 and 7 in appendix), and the protoblasts of the prototroch and velum in annelids and molluscs respectively. Now what are we to say of such a series of contradictions?

Simply that it is impossible, in the present state of our knowledge, to explain them all. But this much may be said: The most striking feature is not the contradictions existing, but the wonderful agreements. The first and third of the facts enumerated state the rule; the second and fourth, the exceptions. The second fact, which is the first exception to the rule, may be explained satisfactorily, as I believe: The ovum, in any stage of development, is an organism complete in itself. Imagine that in any species a new organ is added, or rather, that a diffuse series of structures gains great importance and compactness in the course of evolution. Then, this new structure *may be* represented in ontogeny by a cell. But the form of cleavage is already defined, and each cell has its allotted destiny. The manufacture of a new cell being an impossibility, an old cell must be modified to represent the new organ. In other species, however, the same cell retains its original functions. This hypothetical case is similar, as I shall attempt to show, to the actual case cited above (*viz.*, $a^{2.2}$ in *Nereis* and *Unio*).

The cell ($a^{2,2}$) which in *Unio* supplies the larval mesoblast, is in *Nereis* the left "somatoblast." The larval mesoblast forms, as the name implies, only transitory organs, *viz.*, the isolated muscle-cells, which span the primary body cavity in various directions (V, part III), and the adductor muscle of the larva, which is nothing but a bunch of these myocytes,¹ having nothing to do with the adult adductors (Schmidt, No. 31; Braun, Nos. 6-9). The myocytes are widely spread larval organs in annelids and molluscs; in *Unio* they have increased greatly in number and importance, and have come to be represented in cleavage by a single cell, which thus must differ in fate from the cell of the same lineage in other forms.

If we can speak of an homology of cells of the same lineage in so many annelids and molluscs, it does not, of necessity, follow that the homology must be extended to other forms of the same type of cleavage. I agree with Wilson (No. 64, p. 455) that "the fundamental forms of cleavage are primarily due to mechanical conditions, and are only significant morphologically in so far as they have been secondarily remodeled by processes of precocious segregation." But the fact that the segregation has taken place in the same way in such widely separated instances as have been quoted above, is surely of the widest significance, whether we take it to mean that the ultimate fate of a cell is a function of its position in the cell-complex or not. *It is parallel precocious segregation in different cases that conditions cell homologies.*

Almost every detail of the cleavage² of the ovum of *Unio* can be shown to possess some differential significance. The first division is unequal. Why? Because the anlage of the immense shell-gland is found in one of the cells. The apical-pole cells divide very slowly and irregularly, lagging behind the other cells. Why? Because the formation of apical organs is delayed to a late stage of development. The second

¹ I propose this term "myocyte" for the unicellular muscles spanning the primary body cavity in so many larvae; and functioning as retractors or protractors of the velum or prototroch, *etc.* We have no convenient name in English for these cells, which are known to the Germans as "*Strangzellen*."

² I do not include in this the oblique character of the cleavage or its general form.

generation of ectomeres is composed of very large cells. Why? Because they form early and voluminous organs (larval mantle). The left member of this generation is larger than the right. Why? Because it contains the larval mesoblast. The entomeres are very minute. Why, again? Because the intestine remains rudimentary until a late stage; thus a parallel instance to the apical-pole cells. One can thus go over every detail of the cleavage, and knowing the fate of the cells, can explain all the irregularities and peculiarities exhibited.

These peculiarities of cleavage are all due to the precocious segregation of organs or tissues in separate blastomeres. The order and character of the segregation again are ruled by the needs of the embryo. Thus, one of its greatest needs is the large and powerful shell with which it is provided. The necessity of such provision being made has caused the production of a large shell-gland, which has impressed itself on the segmentation stages as the largest of their blastomeres. I could illustrate the principle in each of the cases just enumerated, but will be satisfied with repeating the introductory sentence of this paragraph in a more special form: The peculiarities of the cleavage in *Unio* are but a reflection of the structure of the glochidium, the organization of which controls and moulds the nascent material.

The larval mesoblast of *Unio* must be regarded as a kind of massed mesenchyme, which has relations to the primary body cavity only. I consider it extremely probable that in other lamellibranchs, and in a wide series of forms besides, ectodermal cells pass into the primary body cavity and function as mesenchyme, producing muscle-cells, or, as in the case of the head kidney of *Nereis*, organs of excretion, or, again, supporting-tissue. Goronowitsch (No. 44*a*) and Miss Platt (No. 56*a*) have both recently certified to the derivation of the bases of certain tissues of the head of vertebrates, hitherto considered mesenchymal, from the epiblast. It may seem fanciful to collate such scattered observations from so widely different classes of animals, but I believe not. On the contrary, it seems to me that the very separateness of these observations gives them a significance all the more apparent. It will help

to convince embryologists that all tissues lying between ectoderm and entoderm are not, of necessity, either mesothelial or mesenchymal, in the Hertwigian sense, and that the occurrence of other elements is not isolated. It does not follow, therefore, that we must deny the homology of the mesoblast throughout. On the contrary, the tendency of the work being done, both on Vertebrata and Invertebrata, is to demonstrate that a portion, at least, of what was previously called mesoblast, is strictly homologous, both in origin and fate, within the limits of the Vertebrata and Invertebrata, respectively. When, for instance, we see that, in a widely varying series of Annelida and Mollusca, the mesoblast is derived from a cell of identical lineage, we must grant that a new and strong proof of homology is adduced. When, on the other hand, we meet with a form like *Unio*, where another conspicuous source of mesoblast is found, we shall decide on *a priori* grounds that such a source probably exists in other forms, but that it is comparatively inconspicuous.

Applying the test of observed facts to this decision but confirms its justice. Ziegler (No. 67), for instance, says, in speaking of the mesenchyme of *Cyclas Cornea* (p. 531), "Es ist mir daher nicht unwahrscheinlich, dass an bestimmten Stellen des Ectoderms Mesenchymzellen vom Ektoderm aus entstehen." Here is another lamellibranch in which it is "not improbable" that the ectoderm contributes to the mesoblast. What is the fate of these "fraglichen Zellen"? They form the single muscle-cells (*Strangzellen*) which are so numerous in the larvae of lamellibranchs. Now, as I shall show, the same cells in *Unio* are derived from the larval mesoblast, also the source of the larval adductor muscle, which has nothing whatever to do with the adductors of the adult (F. Schmidt, No. 31; Braun, Nos. 6 to 9); it is, in fact, nothing but a bunch of *Strangzellen* associated for a common end. I shall speak of this in more detail in the third part of my paper. Stauffacher, the last author on *Cyclas*, seems to regard ectodermal participation in the formation of the mesenchyme as very probable. Lankester (No. 54) is very positive about the derivation of many of the "branching cells" within the segmentation

cavity of Pilidium from the ectoblast. I must call attention to Goette's figures of Anodonta, in which scattered mesenchyme cells are shown in the place occupied by larval mesoblast in Unio, and which could hardly have come from the teloblasts of the mesoderm. Compare, also, the position of the dissociated mesenchyme cells in front of the archenteron in Cyclas with the position of the larval mesoblast in Unio (text, Fig. 7). Fol held that the ectoderm contributed to the mesoblast in pteropods, heteropods, and pulmonates. His observations may still be partly true even though Knipowitsch (No. 49) has seen mesoblast pole-cells in Clione, and Rabl in Planorbis. May there not, too, be some glint of truth in Sarasin's wholesale deduction of mesoblast from ectoderm in Bithymia tentaculata, in spite of the fact that Erlanger (No. 43) has seen the pole-cells of the mesoblast, and has traced them back to the posterior macromere? Wilson has traced back the head-kidneys of Nereis to two ectoblastic cells; Kleinenberg (No. 48) believed in ectomesoblast for Lopadorhynchus. In fact, it would be wearisome to review all the statements in support of the ectodermal origin of some mesenchymal cells, which one could cull from the literature. Unfortunately, most of the statements are qualified (*cf.*, *e.g.*, Ziegler's remark, *supra*), and doubt has been thrown on the rest. It may be, however, that the pendulum has now swung too far in the other direction. I believe in the complicity of the ectoderm in the formation of the mesenchyme. The coelenterate ancestors of the Mollusca possessed mesectoderm cells of contractile function. It would not be very strange if the undoubted phyletic continuity of ectoderm and mesoderm should repeat itself in ontogeny. (*Cf.* Kleinenberg, No. 48, p. 202, *etc.*)

As to the question of the relation of the embryonic axes to the first and second cleavage planes, it seems to me that too much emphasis has been laid on one point, *viz.*: on the relation which the entomeres bear to the embryonic axes. There is a certain justification for this, inasmuch as the entomeres are as a rule so much larger than the ectomeres. But the fact that the ectomeres are given off in different directions from the entomeres has not entered into account apparently. In Crepid-

ula, in Umbrella, and in Nereis the first cleavage plane is said to be transverse and the second parallel to the future median plane. Why? Because it is found, when the embryonic axes are determined, that the entomeres *B* and *C* are on the right side and *A* and *D* on the left side. But no account has been taken of the axial relations of the ectomeres. In *Unio* the decisive factor was with me the second generation of ectomeres, simply because they are the largest cells. As the four cells in question lie anterior, posterior right and left, I said (No. 21) that the future transverse and sagittal axes were inclined at an angle of 45° to both the first and second planes of cleavage. But in Nereis, Crepidula, and Umbrella the relation of the second generation of ectomeres is exactly the same. In Umbrella there is a fourth generation of micromeres, three of the members of which are entodermal and one (the posterior) mesoblastic. This fourth generation bears the same relation to the embryonic axes that the second does. The primary mesoblast *M* in Nereis, in Crepidula, and *Unio* as well, lies in the middle line behind. On the other hand, in Nereis, Crepidula, Umbrella, and *Unio* the first¹ and the third generation of micromeres have the same axial relations as the entomeres. Briefly expressed: the members of the odd generations of ectomeres, as well as the entomeres are distributed, two each, right and left of the middle line; those of the even generations are placed anterior, posterior, right and left. So that if the orientation be based on the odd generations or on the entomeres, it will be said that the first and second cleavage planes are transverse and longitudinal respectively to the embryonic axes; if based on the second or fourth generations of micromeres, it will be said that the first and second planes of cleavage are inclined at an angle of 45° to both transverse and longitudinal embryonic axes. *Unio* thus agrees completely in this respect with Nereis, Umbrella, and Crepidula.

There are forms, however, in which the relations are reversed.

¹ There is a wheel within a wheel here, inasmuch as the divisions of the first generation of micromeres, being oblique, cause certain of the resulting cells to lie on the longitudinal and transverse middle lines.

That is, in which the entomeres lie anterior, posterior, right and left respectively. If the oblique nature of the cleavage remains the same in these forms as in those above cited, the relation of the various generations of micromeres also to the embryonic axes must be different. The best studied forms which show these relations are Clepsine, Planorbis, and Neritina. In Clepsine (Whitman, Nos. 61 and 62), for instance d^2 (x^1 of Whitman) the neuro-nephroblast, or first somatoblast, does not lie in the middle line at first, but to the right (Whitman, Figs. 26, 33, and 34); for some time its products are asymmetrically arranged, but gradually become shifted into bilateral symmetry. In Clepsine D forms the mesoblast immediately after the budding off of d^2 . There may be some correlation between these anomalies of cytogeny and the reversed relations of the blastomere generations to the embryonic axes. But at present we are unable to explain why, when widely separated forms agree, nearly related species should show reversed relations. The first careful study of the second condition will no doubt throw much light on this subject.

Studies of cell-lineage have an important bearing on the nature of differentiation. Any one who watches a segmenting ovum sees the differentiation of the parts which ultimately compose the adult organism going on under his eyes. It is too soon to say that mere observation of the phenomena accompanying differentiation will teach us nothing of its determining factors. As well say that the mere study of the facts of variation will teach us nothing as to the causes of variation! It is a hopeful sign that baseless hypotheses as to the nature of both these phenomena are giving way to a patient study of the accompanying facts.

A tremendous advance has been made since the time when it was thought sufficient to say, "by a series of rapidly succeeding cell-divisions the ovum is cut up into a great number of segmentation spheres, which arrange themselves in the form of a hollow ball." The first segmentation plane has since been shown often to have a definite relation to the future axes of the body; the various cells are not undifferentiated or equivalent, but destined for definite positions and functions in the larval

body. In other words, the blastomeres of segmentation stages have been shown to be the elements of a mosaic. This is a fact which no amount of argument or experiment can remove.

It remains for us to find out how these parts are made, how put together. Inasmuch as we have not as yet the entrance to the room of the raw materials in the workshop, we must study the products, the blastomeres, as they are being formed and after their formation. We must stop the process at each stage and fix it for the most careful and leisurely of examinations; when we have studied every stage of a division known to be differential, and have analyzed all the observable factors, the relation of the chromosomes in the two resulting cells and in each phase of division; of the asters; of the general cytoplasm; and have found out where the earliest indications of the cleavage manifest themselves; then, if we remember that we are observing but the finishing touches to the most elaborate and delicate of mechanisms, it may be that we can argue with some soundness on the philosophy of differentiation.

Conklin has cited some suggestive facts in this connection (No. 41, p. 33): in *Crepidula* however unequal the division of the cytoplasm may be, there is always an equal division of the asters and of the chromatin. "Yet in those very stages in which the nuclei and asters are equal in size, the lobing of the cytoplasm may show beyond doubt that the division of the cell-body is to be very unequal." However, before the cells have separated, the asters have become proportional in size to the surrounding cytoplasm; soon after the separation, the nuclei become proportional in size to the cell-body. In *Unio* I have observed that the resting nuclei become proportional in size to the cytoplasm, though in the different cells they contain originally the same amount of chromatin. Boveri (No. 37a) has observed that polar bodies in *Ascaris megalocephala* will act in the same way as the female pronucleus when brought under similar conditions. How instructive is his account of the continuity of the form of the chromosomes in the germ-cells alone in *Ascaris*, while in the somatic cells this form is lost from their origin! (No. 37.) Observations of this sort

point out the way for future investigation, and give us good cause for hope that, when we know more thoroughly the phenomena which accompany differentiation, we shall not be so much in the dark as to its determining factors.

For many years unequal cleavage has been explained as due to the arrangement of yolk within the dividing cell. So long as it was considered unnecessary to determine the prospective value of individual blastomeres, hardly any other explanation of unequal cell-division in ontogeny was possible. If the cleavage of an alecithal ovum was unequal from the start, *e.g.*, the lamellibranch ovum, a satisfactory explanation was ready to hand: it was due to the inherited effects of the lost food yolk. If unequal divisions occurred at any stage of development, the presence of yolk was sufficient explanation. Similarly yolk was held to retard cell-division by hampering the free action of the cytoplasm. It has even been held that the rapidity of cell-division is proportional to the concentration of the protoplasm. It is of course true in many instances, that unequal cleavage is due to the presence of yolk, but this is, nevertheless, only one phase of a more general law:— Unequal cleavage is conditioned by the constitution of the segmenting ovum, and always means the precocious localization of an organ or set of organs in the larger cell. This organ may be the entoderm, in which case it is usually accompanied by yolk; but the inequality of the first two cells in the annelids and molluscs is the earliest visible indication of another differentiation, the larger cell containing the two somatoblasts. The more precocious the differentiation of the organs of the somatoblasts, the greater the difference in the size of the cells (*cf.* Unio). The two cells may be equal in size when the organs in question are not precociously developed. The same principles suffice to explain unequal divisions throughout the cytogeny. This of course traces back unequal cleavage to protoplasmic structure and is in agreement with Watasé, who says (No. 60, p. 294): “The cause of unequal cleavage in the various cases which we have examined appears to me to be an internal one due to the peculiarities of the particular protoplasmic structure which composes the segment or segments.”

What determines the direction of the spindle and hence of the cleavage? Here again we cannot get back of the organization of the cell. No mechanical explanation will suffice. Let us look for a moment at the cleavages of *X*: The first position of the spindle is on its left side; the second position on the right side

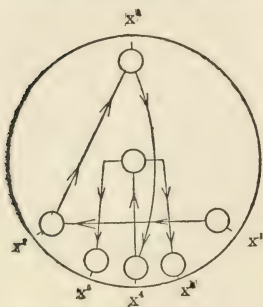


FIG. 2. — Diagram of the Cleavages of the First Somatoblast X.

each one. Finally the nucleus stops in the centre of the cell and a perfectly bilateral spindle (the fifth) is formed (Fig. 44). Why does it stop there? Is it because its environment has changed? If so, the change is such as to elude the closest scrutiny. In fact the cell is a builder which lays one stone here, another there, each of which is placed with reference to future development.

III. GASTRULA TO GLOCHIDIUM.

(a) *Gastrula to Young Larva.*

I. ECTODERMAL ORGANS.

The formation of the shell-gland has already been described. Immediately afterwards the lumen is filled with a transparent, refringent substance, the first indication of the cuticular shell. A rapid evagination of the capacious gland now ensues. At the same time there is a thinning out, which proceeds more rapidly in the centre than at the sides. This is, of course, due to the extension of the area covered by the same cells. When

(Figs. 25 and 27); the third in the middle line towards the apical pole (Figs. 33–35); the fourth in the middle line towards the vegetative pole (Fig. 39). (See accompanying diagram.) In none of these cases does the spindle occupy more than a fraction of the diameter of the blastomere in question. The nucleus has been wandering through the cytoplasm from one side to the other, from the front to the back, stopping at various stations, and giving off a cell at

the evagination is completed the shell appears in the form of a very delicate, transparent, cap-shaped cuticle, covering the whole of the shell-gland area, and which is derived from the above-mentioned refringent secretion of the shell-gland.

As has often been noted, the shell is not at first bivalve; nor is it so when the gland has evaginated, and the dorsal line begins to undergo lateral compression (Figs. 68 to 71). The cuticular plate is merely bent over the dorsal surface, and adheres on each side to cells which represent the border of the evaginated gland, *and which are sharply marked off from their neighbors* (Pl. V, Figs. 75 and 76). The limits of the shell-gland are thus the limits of the shell. It will be noticed that the nuclei of the cells to which the margin of the shell is attached lie very near the surface, from which I conclude that they play an active part in the secretion and growth of the shell. It is not until some time later that the hinge makes its appearance.

The cells lying immediately beneath the shell become progressively thinner in proportion as the surface covered by the shell increases. Finally they become reduced to a mere film of protoplasm in which the nuclei produce swellings (Pl. V, Fig. 75; Pl. VI, Figs. 87 and 91, *etc.*). The growth of the larval shell takes place wholly from the cells which lie along its margin. As the cells which lie beneath the shell thin out, this region of the embryo becomes perfectly transparent (Pl. V, Fig. 79).

Great changes in the form of the embryo take place at this time. The general character of the changes in question can be seen by a comparison, in serial order, of the figures from 64 to 79. The most important elements of the change in form are two: On the one hand, the expansion of the shell-bearing region and the accompanying bilateral compression (*cf.* Figs. 62 and 68, with Figs. 76 and 80); and, on the other hand, the expansion of the anterior portion of the embryo; that is to say, the portion in front of the blastopore and of the shell-gland. (Compare this region in Figs. 66, 69, and 79.)

About midway between the blastopore and the cells of the head vesicle (*h.v.*) a slight depression becomes visible at the

time of invagination of the shell-gland (Fig. 69, *o.p.*). The bottom of the depression is occupied by numerous small cells very closely appressed, so that the cell-boundaries are seen only with difficulty. In whole embryos the region is marked by the cluster of active nuclei formed here. Flemming applied the name "Mittelschild" to this region in acknowledgment of his ignorance as to its fate; later he conjectured that it might form the oesophagus, but he was so uncertain about it that this can be regarded only as a lucky guess. Rabl at first took it to be the basis of the oesophagus: "Später aber überzeugte ich mich dass diese viel weiter oben am Vorderende des Körpers . . . entsteht und sich schon sehr frühzeitig mit den Entodermzellen verbindet" (Rabl, *l.c.*, p. 366). Schierholz was the first to clearly recognize its true significance. He applied to it the name "Mundschild," which I shall translate *oral plate*. The oral plate is destined to form the oesophagus, but it is a very long time before it does so and comes into function. Its place of formation is some distance in front of the anterior end of the blastopore, with which it cannot have had any connection at any previous stage. Later on, however, it moves bodily backwards and meets the ventral plate which at this stage marks the anterior border of the primitive mouth. (In another place I give the details of this process.)

This brings me to the consideration of *the closure of the blastopore and the extension of the ventral plate, two processes which go hand in hand*. In its early condition the blastopore has a considerable *antero-posterior* extent, and is quite wide (Pl. V, Figs. 67 and 68). Fig. 67 illustrates the important point that even at this early stage *the anterior and posterior lips of the blastopore are bounded by cells of very different appearance*. In later stages the distinction becomes much more evident (*cf.* Pl. V, Figs. 69 and 73). The posterior lip of the blastopore is formed by the small cells of the ventral plate (Pl. V, Fig. 67), which was elsewhere (p. 25) shown to be derived from the first somatoblast. The anterior lip is formed by the high columnar cells of the future larval mantle. The mesoderm teloblasts lie just behind the posterior lip. If Fig. 69 be compared with Fig. 67 it will be seen that, whereas the

same differences exist between the anterior and posterior lips, they are now near together. But the mesoderm teloblasts no longer lie immediately behind the posterior lip of the blastopore; they are separated from it by a wide space. It therefore follows that the blastopore has closed from behind forwards. How has this closure taken place? *By the forward growth of the ventral plate, which eventually meets the large columnar cells marking the position of the anterior blastoporic rim* (Pl. V, Figs. 69 and 73). Thus a plate of cells is established, extending from the hinder limit of the shell-gland to the anterior limit of the blastopore.

It is this plate of cells which becomes covered with cilia in embryos of Anodonta, the action of which causes a rotation of the embryo on its antero-posterior axis. In *Unio* such a rotation does not take place. It has, hence, been concluded by some (Rabl, No. 25) that the cilia are absent from this region. Others assert their presence (Schierholz, No. 30). With comparatively high powers of magnification ($\times 600$) I have been able to see very distinctly that this region is covered with extremely fine and active cilia in embryos of *Unio*. Small particles within the egg membrane which came in contact with the ventral plate were immediately swept away, and always toward the mouth region.

Considerable discussion has taken place as to the morphology of this ciliated tract. Thus it has been supposed to represent a rudimentary velum. The last idea published is, that they represent either the ventral or post-anal cilia of other lamellibranch embryos (Korschelt and Heider, No. 51). The question is of the morphology of the region bearing them. The region is that of the ventral plate, which, as I have already said, forms the foot and pedal structures, and, in addition, the post-anal region. The cilia in question, then, are homologous with those on the ventral surface of other Molluscan embryos. I shall have to postpone proof of this to a still later section.

A glance at Fig. 69 reveals the fact that just beneath the anterior end of the shell-gland there are large cells provided with large nuclei, each of which possesses a well-marked nucle-

olus. A surface section including these cells is shown in Fig. 71. They are six in number, the nuclei large and transparent, with usually a single very prominent nucleolus. Rabl has figured these cells. A comparison of his Fig. 29 with my Fig. 70 will leave no doubt as to their identity in the two cases. They have, of course, been seen by Schierholz, who has seen all that Rabl did. Rabl says of them (No. 25, p. 328): "An der dem späteren Hinterende entsprechenden Körperseite machen sich zu dieser Zeit drei durch ihre ausserordentliche Grösse und ihre kugelige Form von allen anderen Ectodermzellen auffallend abweichende Zellen bemerkbar"; and further on, p. 370: "nach meinen Beobachtungen entsteht diese Drüse" (thread-gland) "durch eine, zwischen drei, am Hinterende des Körpers gelegenen Zellen, auftretende Einstülpung des Ectoderms." He goes on to say that he cannot determine with certainty whether or not these three cells are derivatives of those mentioned before. It will be noticed that Rabl speaks of three cells, whereas I have figured six. As a matter of fact, three of these are more conspicuous in surface views than the other three.

According to my observations, Rabl is right in deriving the thread-gland from the region of these cells, but wrong in attributing its formation to invagination; *it is only one of these cells which forms the thread-gland proper*, and that is the one shown in the centre of the six (Pl. V, Fig. 71). Though smaller in superficial extent, this cell is really as large as those surrounding it, for it is much deeper. The six cells in question become more or less vacuolated; the nucleus of the central cell migrates to its inner end, the greater part of the protoplasm following it; finally, almost all of the cell lies within the primary body-cavity, but is still connected with the spot it formerly occupied by a strand of vacuolated protoplasm. The vacuoles run together, and form a lumen running from near the nucleus to the exterior. Almost immediately a refringent cuticular lining of the lumen is formed. The development of the gland may be traced through Figs. 73 and 74. The other five cells of the complex later surround the opening of the gland (Fig. 81; cf. Fig. 43 of Rabl). These

five cells persist in their characteristic position, and without any alteration of nuclear structure, until the glochidium is fully formed.

Fig. 74 is a part of Fig. 73 much more highly magnified. The nucleus occupies the inner end of the gland, and is identical in appearance with those between whose cells the gland opens. The lumen penetrates to within a short distance of the nucleus, and then disappears. Rows of granules may be seen radiating from the nucleus to the inner end of the lumen. The position of the gland, the character of its nucleus, and the presence of five nuclei only round its opening in place of the six original cells, make certain its derivation from the central cell of Fig. 71. The terminal nucleus persists for some time longer (Fig. 79), but gradually dwindles and disappears. During its existence it no doubt controls the growth in length of the gland, but after its disappearance the further growth seems to be supported by the five large cells around its opening.

Briefly, the further history of the thread-gland is as follows: It grows backwards beneath the hinge line until it reaches the posterior end of the body; then turns down, and passes on the right of the entodermic sac to the large cells of the larval mantle, which it enters at the angle of the shell. The apical nucleus has by this time disappeared. The gland continues its growth through the cells of the larval mantle, until it reaches its anterior end, when it turns dorsad to its opening beneath the anterior end of the hinge line. This is the extent of the gland in the stage represented in Fig. 82 (Pl. VI). In later stages a still greater length is attained. After the invagination of the mantle the gland takes two or three turns around the adductor muscle in the right valve of the shell.

How is the thread formed? It is hardly conceivable that such a long (10 to 15 mm. *Forel*) and strong structure should be formed by the activity of a single cell. Nor is it, in spite of the fact that the gland arises from a single cell. The thread must be thought of, not so much as a secretion into the lumen of the cell, as an actual metamorphosis of the substance of the cell (Pl. VI, Figs. 85, 91, *etc.*). When this has begun (in

the stage of Fig. 82) numerous mesoderm cells apply themselves to the gland and completely encase it. The further growth and secretion seems to be supported by these cells. The extrusion of the thread takes place some time before the rupture of the vitelline membrane.

One of the earliest and most natural ideas in regard to this organ was, that it was the homologue of the byssus-gland of other lamellibranchs. Rabl seems to have adopted this idea; he at any rate uses the term byssus-gland. Carrière (No. 10) was the first to show that this view is untenable, both from the position of the organ, and also from the fact that an actual byssus-gland is formed in the parasitic larva. He came to the only tenable conclusion, *viz.*, that it is an organ *sui generis*. As such it is still regarded, and I have found no characteristic which would cause it to rank with other organs elsewhere. But the morphology of the region which it occupies is very imperfectly understood; it is this latter question which I wish to clear up.

Ziegler (No. 67) suggested that the three bladder-like cells described by Rabl just in front of the shell-gland were a rudimentary head-vesicle. In the third volume of their text-book, Korschelt and Heider advance the same idea. This suggestion contains but part of the truth; they are, in fact, but part of a rudimentary head-vesicle. The head-vesicle in molluscan larvae is the region in front of the velum, which passes just in front of the shell-gland and of the mouth on the dorsal and ventral sides, respectively. The homologous region is thus easily defined in *Unio*. It is represented by the larger part of the region in front of *o.p.* (Pl. V, Figs. 69, 73, 79), and of the shell-gland or shell. It is thus seen to be quite an extensive area. The thread-gland opens at its dorsal limit in the middle line. It thus lies very nearly in the course which the velum would take if present, and I was therefore at first inclined to interpret its protoblast, with the similar cells surrounding it (Fig. 71), as cells of a rudimentary velum. However, comparison with Ziegler's figure (No. 67, Fig. 6) convinced me that the cells in question were really cells of a rudimentary head-vesicle. If Ziegler's Fig. 6 be compared with my Fig. 69,

a very striking agreement will be found in this respect. Just in front of the cells of the shell-gland in both cases are large rounded cells with large nuclei (text, Figs. 7 and 8 *h.v.*). In *Cyclas* these cells enter into the formation of the head-vesicle. In *Unio*, the head-vesicle not being developed, we can only regard these cells as a part rudiment of that once important structure.

The cell which forms the thread-gland is thus one of the cells of the head-vesicle. No such use is made of these cells elsewhere and so the organ must retain its rank as morphologically *sui generis*. But is it so physiologically? According to the generally received idea that the function of the thread is merely to assist the larva in attaching itself to its host, it is. It seems to me, however, that this cannot be its sole function. It certainly cannot have been the primitive function; for if it be of such assistance to the larva, it can only be in virtue of the length and strength of the thread secreted, and such an extensive structure could hardly come into existence fully formed. Its primitive function, both ontogenetically and phylogenetically, was probably excretion. Let us see what evidence there is in the actual development. I have already called attention to the rows of granules which radiate from the nucleus toward the lumen of the gland in early stages (Pl. V, Fig. 74). I take these to be an indication of active secretion on the part of the gland.¹ But after the terminal nucleus has disappeared, and this kind of excretion has ceased, matter still continues to be excreted through the gland, only now in the form of a solid substance, the thread. It is not necessary to assume that the function of excretion is lost simply because the products of excretion are utilized. Instances of utilization of waste products (so called) on the part of the animal producing them are by no means rare. This is moreover the only active larval organ which communicates freely with the exterior. If its

¹ It may, perhaps, be worth while to call attention to somewhat analogous function of cells of the prae-trochal region in *Nereis*. Wilson has discovered that two cells lying not far from the apical plate but behind it, move into the cavity of the head vesicle, where they acquire a lumen. He interprets them as head-kidneys; with a certain reservation he it said. These cells are paired, the thread-gland of *Unio* is, however, a median structure.

function be not that of excretion in the strict sense of the word, we would have the anomaly of an active larva without any provision for the excretion of waste products. The waste products in this case take the form of an insoluble substance as an adaptation to development within the strong vitelline membrane where soluble waste products could not but act injuriously. The thread-like form of the waste substance is the mechanical result of the form of the secreting gland. If the thread really assists the larva in attaining its host, this is a secondary and subordinate function.

Of the ectodermal structures of this stage it remains only to consider the rudiment of the larval mantle. This includes all the cells from the blastopore to the opening of the thread-gland. Its lateral extension is well shown in Fig. 79, all of the cells below the shell being included in it. It will be seen that the oral plate lies about in the middle of the area in question. The cells are cylindrical with the nuclei about the middle of their height. They resemble each other throughout the whole extent of the area, excepting in one narrow strip in the median line extending from the opening of the thread-gland to the oral plate (Fig. 80 *s.c.*). The cells in question were noticed by Flemming and called by him suture-cells ("Nahtzellen"). They are very narrow long cells and mark the line of division of the right and left halves of the mantle.

About this time (Figs. 79 and 80) appear the bristles which later become such a characteristic feature of the glochidium. There are but three pairs of these organs at this time (Figs. 79 and 80). Each is a little bunch (three to five in number) of stiff hairs born by a special cell. The position of these six cells is: one on each side of the thread-gland; one on each side of the oral plate, and one on each side of the anterior end of the ventral plate. A fourth pair is soon formed beneath the first pair mentioned.

2. MESOBLASTIC ORGANS.

We left the mesoblast in the stage in which eight cells are formed: four of the primary (*M*) and four of the larval mesoblast (*Y*) (Figs. 64, 65, 72, *etc.*). The cleavage of the parent

cells of the primary and larval mesoblast differs from the first. The divisions of the primary mesoblasts are teloblastic, those of the larval mesoblasts bear no such definite relation to the embryonic axis, but are irregular. The primary mesoblast cells adhere and act more like a mesothelium. The larval mesoblast cells are mesenchymal in their lack of coördination. This difference in the two kinds of mesoblast enables one to distinguish them throughout. In a considerably later stage (Pl. V, Fig. 69) the primary mesoblast forms definite germ-bands with terminal teloblasts. In front of the germ-bands lie the elements of the larval mesoblast.

At first sight there appears to be a considerable gap between the stage of Fig. 69 and the succeeding stage of Fig. 73, but the gap is more apparent than real, and is due to two changes. First the eversion of the shell-gland and secondly the appearance of the larval adductor muscle and myocytes. Even in the stage of Fig. 69 the adductor muscle and myocytes are foreshadowed; thus a myocyte is seen stretching from the oral plate to the entodermic sac.

The position of the mass of the larval mesoblast cells marks the position of the future adductor muscle. Moreover, and this is a fact of some importance, the nuclei of these cells resemble the nuclei of the early adductor muscle cells. Again, the primary mesoblast is a compact fundament in the earlier stage (Fig. 69); so is it in the latter stage. Here, however, transverse sections are necessary for its demonstration; two of these are shown in Figs. 77 and 78, taken in the planes marked by the lines (77) and (78) in Fig. 73. The most posterior section (78) shows the mesoderm teloblasts still plainly recognizable; they have, however, shifted their position to the sides of the entodermic sac (*cf.* Fig. 69). The primary mesoblast in front of the teloblasts is in the form of stout wings of cells stretching on each side from the entodermic sac to the walls of the body. In the place previously occupied by the elements of the larval mesoblast we have the larval adductor muscle and the myocytes. When, in addition, the above mentioned similarity of nuclear structure is remembered, it is impossible to resist the conclusion that the larval adductor

muscle and the myocytes have been formed at the expense of the larval mesoblast.

The mere fact of the common origin of the adductor muscle and the myocytes proves them to be formed from homologous elements. This fact, of course, includes their histological identity in early phases of development. As both F. Schmidt (No. 31) and Braun (Nos. 6-9) state definitely that no continuity between the adductor muscle of the larva and those of the adult exists, the conclusion that the larval adductor is merely an accumulation of myocytes is unavoidable. Thus another characteristic glochidium structure is brought into direct line with homologous parts elsewhere.

It seems almost superfluous to add that the distribution of the cells of the larval mesoblast throughout the primary body cavity does not convert the latter into a true coelom. This is, however, what Rabl has affirmed. Study of the post-larval development has shown that the true coelom appears much later, as the pericardium.

The larval muscle is composed at first of much elongated cells, with very granular cytoplasm and round nuclei (Figs. 75 and 76), each of which is provided with two distinct nucleoli. Later on the arrangement of the granules becomes very regular; the nuclei become oval with their long axis in the direction of the length of the cell; they are then drawn out into a rod-like shape and sometimes take up nearly half the length of the muscle fibre. This condition is reached in the stage of Fig. 91. This section, however, does not show the full length of any of the nuclei. In still later stages the muscle cells show a longitudinal fibrillation. Histological differentiation is then complete. I should add, however, that there is either a fragmentation or great shrinkage of the bacillus-like nuclei. For in the fully formed glochidium the muscle nuclei are quite minute.

Structures very similar in appearance to the "Strangzellen" are formed from the primary mesoblast; they are two stout wings of cells which run from the entodermic sac to the walls of the body. Not only are these different from the myocytes in origin, but also in their fate. They soon fall into a clump

of small cells (Schmidt, No. 31 and Schierholz, No. 30), which are destined to form the pericardium, nephridia, and perhaps other mesoblastic structures. At no stage of their embryonic history are these cells contractile.

To return to the myocytes. Schierholz has distinguished six on each side; but as of these, two pairs belong to the primary mesoblast, there are but four pairs of *myocytes* constant in position. The two most prominent pairs are shown in Figs. 75 and 76; the relative position of these will be better understood after reference to Fig. 73, in which the planes of the sections are indicated. Another pair passes from the oral plate to the entodermic sac (Fig. 69); and, sometimes at least, there is still another pair, partly indicated in Figs. 73 and 74, attached on the one hand just beneath the aperture of the thread-gland and on the other just in front of the oral plate. A very conspicuous strand is that shown in Fig. 79, running from the entodermic sac to the shell near the postero-dorsal border of the adductor muscle.

What is the function of these cells? Schierholz assigns to them an important mechanical part in development. Thus by their steady and slow contraction they produce invaginations, *e.g.*, the lateral pits, or the larval mantle; again they shift the relative position of the parts. These ideas are on a par with his suggestion that the oral plate is produced by the pressure of the overlying polar bodies (which as a matter of fact lie slightly in front of this plate). One cannot deny the originality of this view of the mechanics of development; neither can one accept it. The theory of unequal growth as the cause of invagination and kindred phenomena in development was never better illustrated than in the Unionidae. As a matter of fact, wherever in the embryo invaginations occur, clusters of active nuclei are to be observed (*cf.*, *e.g.*, the oral plate, Fig. 75, or the lateral pits, Fig. 95). It is true that myocytes are attached to the region of the oral plate; it is however quite common for the larval oesophagus to possess such attachments (*cf.* Hatschek, on *Teredo*). As for his theory of the lateral pits, the "Strangzellen" in that neighborhood are not myocytes, but derivatives of the primary mesoblasts.

The actual function of the myocytes is the same as in other animals: *i.e.*, to carry out temporary movements of parts. Some of them may be compared, though not perhaps homologized, with the retractors of the velum in *Teredo* for instance. The pair shown in Fig. 76 is doubtless that which functions later on in the movement of the hooks of the shell. Before the invagination of the larval mantle they all act as retractors of the soft parts.

3. ENTODERMAL ORGANS.

We left the entoderm in the form of a small sac communicating by a comparatively wide mouth, the blastopore, with exterior (Pl. V, Figs. 66, 68). In the next stage the blastopore is practically closed by the forward growth of the cells of the ventral plate area, and the entoderm is now represented by a small clump of cells lying in contact with the ectoderm. The dorsal and ventral lips of the blastopore meet, but do not fuse, so that it is possible to recognize the anterior end of the blastopore throughout. The entoderm generally assumes the form of a sac in the stage of Fig. 73 (*cf.* Fig. 77), but at other times no lumen is discernible. This is but an example of the usual variability of rudimentary structures.

4. SUMMARY.

In this section we have followed the development of the gastrula into the young larva. Before passing on to its transformation into the glochidium I shall briefly describe the young larva, comparing the names which I have used for the various parts with those already in use, to serve as a point of departure for the next section. In side view the young larva is roughly quadrangular; in transverse section, or end view, triangular (Figs. 79, 80). The straight hinge-line extends along the whole of the dorsal surface. Each valve of the shell covers about two-thirds of its side; the anterior, posterior, and ventral edges of the shell are curved (Fig. 79). The cells lining the shell are extremely flat; so much so, that the nuclei produce swellings. The ventral surface from the margin of the shell is formed of large columnar cells. About the centre of the

ventral surface is a shallow indentation, the floor of which is formed of small, closely appressed cells (oral plate; "Mittelschild," Flemming). Just beneath the anterior angle of the valves of the shell is the opening of the thread-gland, surrounded

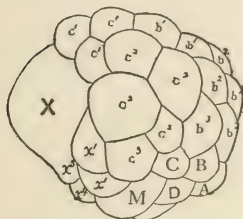


FIG. 3.— Fifty-cell stage of *Unio* from the right side.

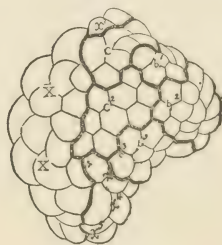


FIG. 4.— Gastrula of *Unio*.

by five large cells (Figs. 80 and 81). The median line, from the opening of the thread-gland to the oral plate, is occupied by long cells drawn out in an antero-posterior direction. These are the suture-cells (Nahtzellen of Flemming), to which more attention will be directed in the next section. Below the posterior angle of the valves of the shell in the median line is a plate of ciliated cells; this I have called the ventral plate (Wimperschild of Flemming). These cells extend forward, and stop suddenly at the larval mantle cells (the columnar cells of the ventral surface already referred to). The place where the ventral plate meets these cells is the anterior limit of the blastopore. Lying within the ectoderm at this spot is the entodermic sac (Vorderwulst of Flemming), with mesodermic cells attached (Figs. 73, 77, and 78). The teloblasts of the mesoderm have now moved from their former position in the angle of shell-gland and ventral plate to just behind the entodermic sac. Two stout wings of cells pass from here to the lateral ectodermic walls (Pl. V, Fig. 77) (Seitenflügel of Flemming). Within the primary body cavity are numerous elongated

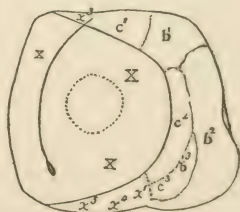


FIG. 5.— Young Larva of *Unio*.

mesodermic cells (myocytes; products of the larval mesoblast), some of which show a paired arrangement (Strangzellen of authors). Stretching across the primary body cavity from one shell valve to the other, nearer the anterior than the posterior end, is the larval adductor muscle, likewise a product of the larval mesoblast; dorsal to this, and running parallel to the hinge-line from the posterior end of the primary body cavity to open anteriorly between the five large cells already noticed, is the large unicellular thread-gland.

The accompanying text figures (3, 4, and 5) will make the relations of the areas more readily referable to the cleavage stages. It of course goes without saying that the areas in the second and third figures are only approximately correct in outline. The first figure is an actual reproduction of Fig. 47 (Pl. IV).

(b) *Transformation of the Young Larva into the Glochidium.*

Perhaps the simplest way of describing the transformation into the glochidium will be, first, to describe the glochidium, and then to ask how this form is derived from that of the young larva already described. Figs. 92 and 93 illustrate this description.

The glochidium larva of *Anodonta* possesses two triangular shell valves joined by their bases at the hinge-line. The valves are quite thick, strong, and brittle, and pierced by numerous fine pores. At the apex of each valve is a strong hook (provided with numerous teeth), which is quite different in appearance in *Unio* and *Anodonta*, being much stronger in the latter form, which I have figured. These hooks are joined to the valve proper by a hinge, and are moved by special muscles (myocytes). Each valve is somewhat spoon-shaped, and the cavity is lined by the larval mantle, consisting of large, flat vacuolated cells. The curve of the anterior edges of the valves is considerably greater than that of the posterior edges; the hinge-line straight, and of considerable extent. The valves are united by a strong internal ligament. The adductor muscle is very powerful, and, as in the young larva, is nearer the anterior than the posterior end. The larval mantle bears four paired

tufts of stiff, sensory hairs, arranged in a very characteristic manner. Three pairs lie just beneath, and within the powerful hooks; these three form the angles of a right-angled triangle, the base of which is parallel to the transverse plane of the larva, the apex being directed anteriorly. The fourth pair lie on either side of the opening of the thread-gland. These are, undoubtedly, the four tufts described for the young larva; but how different in their relative positions! As in the young larva, each tuft is borne by a single cell, the base of which is elongated in a peculiar manner, to be described more fully later on.

Near the posterior angle of the valves are two ectodermal pits—the lateral pits. Between them, beneath the ectoderm, is the entodermal sac. Behind this lie the lateral wings of mesoderm cells. The ventral plate occupies the whole of the posterior median region as far forward as the oral plate, which has now assumed the form of a stomadeal invagination. Just in front of the oral plate is the opening of the thread-gland, from which the long, much-tangled larval thread has been extruded.

The transformation of the young larva into the glochidium is attended by a series of shiftings and displacements of cells and groups of cells, which make this part of the development extremely difficult to follow. Flemming is the only author who has described these processes in detail; and indeed his description leaves little to desire in some ways. But Flemming was so uncertain as to the morphological meaning of the larval parts that he gave them all special names: “Wimperschield” = ventral plate; “Vorderwulst” = entodermic sac; “Mittelschild” = oral plate. It is due to this failure on the part of Flemming to recognize the homologies of these various parts that the apparent neglect of this part of his work is due.

The early appearance of the four paired tufts of hairs is of great assistance in following these changes. The arrangement of the sensory hairs on their first appearance has already been described. Their final arrangement may be seen in Fig. 93. To recapitulate: At first they are arranged in a row on each side, as follows: one tuft to the side of the thread-gland aperture, and a second tuft a little below this; the third lies just above the oral plate, and the fourth to the sides of the ento-

dermic sac.¹ During the metamorphosis the first two pairs move backwards with the thread-gland nearly to the oral plate. They thus come to be associated with the third pair of sensory hairs, which lie near the oral plate. When the invagination of the larval mantle takes place it is these three tufts which lie beneath the shell-hooks on each side. By this time the oral and ventral plates have grown together, and the fourth pair of sensory hairs now lies a little in front of the oral plate, and to the sides of the aperture of the thread-gland.

From the posterior angle of the valves of the shell to the oral plate—which is now assuming the character of a stomadeal invagination (in February glochidia of Anodonta)—the whole median surface is formed by cells of the ventral plate. The further growth forward of this plate is what causes the anterior displacement, in parasitic glochidia, of the oral plate to its definitive position. The foot—which is formed in this region—is then derived from cells of the ventral plate. It of course goes without saying that during these shiftings the larval mantle has invaginated. Having thus outlined the metamorphosis, it now remains to treat of each stage in detail.

The most striking difference between the young larva and the glochidium is the bifid condition of the mantle in the latter as contrasted with its unpaired condition in the former. It has been known since the time of Flemming's paper that the difference is established by the invagination towards the dorsal line of the whole ventral surface of the young larva along the median plane. But while this has in general been known, the histological changes which must accompany such a stupendous transformation have never been described. These are illustrated in Figs. 83 to 91. The preparation consists, first, in the establishment of a line of suture-cells (Figs. 80, 82, and 86) which divides the basis of the larval mantle into two halves, from the thread-gland to the oral plate; and, second, in the vacuolation of the cells which are to invaginate. Invagination

¹ This differs from Schierholz's description of their original position. It may be, however, that he overlooked their earliest appearance, when they are seen with difficulty. Schierholz described three pairs as lying near together, just beneath the edge of the shell, at the transverse level of the oral plate. This is not their original position in *Unio*.

commences, as might be expected, along the line of the suture-cells, and is at first most active near the thread-gland (Fig. 82). It is accompanied by very pronounced changes of form on the part of the invaginating cells. The greater mass of the protoplasm migrates to the inner end of the cells (Fig. 85); the nucleus accompanies it—a very usual appearance in large invaginating cells; the cells then roll up and in towards the shell, which thus comes to be lined by two layers on each side (Figs. 87 and 90): first, the protoplasmic layer already spoken of (which is very intimately attached to the shell); and, second, the cells of the invaginated mantle. Beginning, as I have said, in front, the invagination passes backwards, in proportion as the oral plate travels towards the ventral plate, which, on its part, moves forward to meet the former. *The rest of the mantle rudiment is thus divided into lateral halves and invaginates under the same appearances, carrying with it the median ventral and oral plates, which have now met and occupy the whole of the posterior region.*

The vacuolation of the larval mantle cells begins in quite an early stage—about the stage of Fig. 79. Flemming has figured and described the appearance of these cells at this time. The vacuolation, which is not at first very marked, soon becomes more and more exaggerated (Fig. 85). Indeed, it seems as though there was an active effort on the part of the cells in question, with a given amount of protoplasm, to cover the greatest space possible. The transition from the compact columnar cells of Figs. 76, 77, and 78, to the flat, much-vacuolated cells of Figs. 84 and 93 is most striking.

The suture-cells are well seen in Fig. 90 and in section in Figs. 85, 86, and 87. They are long, spindle-shaped, deeply staining cells, with rod-like nuclei; in cross section they are wedge-shaped (Pl. VI, Fig. 85).

During the invagination of the mantle, the thread-gland has shifted its position backwards along the line of the suture-cells, and now lies just in front of the oral plate. Although the displacement of the thread-gland takes place along the line of the suture-cells, yet I should hesitate to attribute any active share to these cells. They seem rather to be the preformed

path of displacement, and serve to separate the halves of the larval mantle as well. Schierholz has figured a muscle-cell connecting the thread-gland with the oral plate, and attributes to it the function of causing this displacement.

The ventral plate goes through some interesting changes (to which I have already referred) about this time. Figs. 83 to 89, representing sections in various planes through the stage of Fig. 82, illustrate the description. The ventral plate grows past the posterior margin of the larval mantle (which represents the anterior end of the blastopore) and towards the oral plate (Figs. 83 and 84), which is at the same time moving backwards through the larval mantle. At this time the oval plate can be recognized only as a cluster of deeply staining nuclei (Fig. 84), all traces of its previous pit-like condition being obliterated by the great expansion of the cells of the larval mantle. A tongue of cells of somewhat similar appearance to the ventral plate at the opposite end of the embryo might lead to the belief that similar processes of development were responsible for the two structures; and hence that the appearance of free, forward growth of the ventral plate was illusory. But that this is not the case is proved by horizontal sections (Figs. 86 and 87). These show that at the anterior end of the embryo (*i.e.*, directly opposite the ventral plate) there is a protrusion of the cells of the larval mantle without the shell, caused of course by the violent contraction of the adductor muscle on the addition of killing reagents. There is nothing of this sort to be seen at the posterior end. It is the section of the protruding cells which is seen in sagittal section at the anterior end. (*Cf.* lines of section in Figs. 84 and 87.) I have hence been forced to conclude that there is an actual growth forward of the ventral plate above the cells of the larval mantle. The oral plate has been moving backwards through the larval mantle at the same time. During the invagination of the mantle the two structures meet and unite (Fig. 88).

The mantle cells have rolled away to the side; and hence the whole of the median portion of the glochidium, from the oral plate to the posterior angle of the shell, is formed from the ventral plate (Fig. 93). According to the united testimony of

those who have studied the post-embryonic development, the anterior part of this area is the basis for the formation of the foot and pedal structures.

Schierholz derives the rudiment of the foot from the median portion of the cells of the larval mantle lying between the oral plate and the anterior end of the ventral plate. That I am unable to agree with him goes without saying.

It is practically certain that the anus forms behind the anterior limit of the blastopore, but still within the limits of the area originally occupied by its posterior portion.

It will suffice to merely mention the lateral pits (*cf.* Figs. 89 and 93 to 97) lying at the sides of the foot-fold, as we may now call the area of the ventral plate. They are covered with active cilia, which are in direct continuity with the cilia of the foot-fold (Fig. 93). The structure of the walls is shown in the sections (Figs. 94 to 97). Schierholz and Schmidt derive the gill-filaments from the outer walls of these pits. Within the pits, according to Schierholz, lie two or more rounded cells which he regards as the basis of the otocysts. I have sometimes seen such cells in the stage of Fig. 79 lying on the surface near the anterior end of the ventral plate, but it seemed to me that they did not persist. In any case it is difficult to see how they could represent the otocysts.

The cerebral ganglia have begun to form in some glochidia of *Anodonta* in February. A section through their rudiment is shown in Fig. 94, which is taken about 22 μ . in front of the stomodaeum.

The bristles which lie on each side of the thread-gland and beneath the hooks of the shell have been considered sensory by all authors who have mentioned them. I found that when the glochidia were left for some time in a weak solution of methylene blue the cells bearing these bristles were the only ones in the embryo which took the stain. After fixing with picrate of ammonia, long, stained protoplasmic processes of these cells could be traced for some distance beneath the larval mantle. In Fig. 86 I have shown the course of the processes of the lateral bristle-bearing cells. In no case was I able to make out any coördinating structure with which the processes

were connected, though one can hardly doubt that such a structure exists. Flemming and Rabl have figured somewhat similar but shorter protoplasmic processes to the bases of these cells. Their reaction to methylene blue seems to me fresh evidence of their sensory nature.

I do not include a detailed description of the cells in question; for that has already been done by Flemming and Rabl. I shall merely call attention to the conical form of these cells and their elevation above the surrounding surface (Fig. 92). They are supposed to transmit to the adductor muscle the stimulus which causes its contraction. The necessary stimulus might of course come from contact with the prospective host, in which case the contraction of the muscle would force the hooks into its skin, thus securing the requisite attachment (Schierholz).

Mesoblast.

In the stage of Fig. 82 and presumably somewhat later, the teloblasts of the primary mesoblast are still recognizable. The remainder of the primary mesoblast has fallen into a clump of small cells, which take up a position behind the entodermic sac as the latter moves forwards (Fig. 84). In the glochidium the mesoblast is very distinctly paired and lies in contact with the posterior walls of the lateral pits (Figs. 89, 94, and 97), stretching to the posterior end of the embryo on each side. A special wing of the mesoblast may be seen on each side behind the lateral pits. In well stained specimens this portion of the mesoblast shows but few clear nuclei with distinct nucleoli (Fig. 93). According to Schmidt, these cells are the fundaments of the organ of Bojanus (the nephridia).

Some of the myocytes are specially modified as retractors of the hooks (Schmidt). The others are attached to the larval mantle and shell, and serve to keep the former in varying degrees of approximation to the latter.

Entoderm.

In embryos of *Unio* of the stage of Fig. 82 the entoderm no longer forms a sac, but has become a mass of cells (Figs. 83

and 84). It is already beginning to stretch forward towards the oral plate above the cells of the larval mantle. It is no doubt the mechanical cause of the splitting of the larval mantle, which permits the ventral plate to come in contact dorsally with the entoderm sac and to fuse anteriorly with the oral plate. In the glochidia of *Unio*, which do not as such reach so advanced a stage of development as those of *Anodonta*, the entoderm remains in this state till the post-embryonic development begins (*cf.* Fig. 89). In those glochidia of *Anodonta*, however, which have wintered in the maternal gills, the entoderm has already begun its differentiation. It has taken on the form of a sac which in one case ran through seven sections of $7\frac{1}{2}$ mm. each (Figs. 95-97). Lateral expansions in the middle of its course I took for the liver diverticula from comparisons with Schmidt's sections of parasitic larvae (Fig. 96 *l.c.*). Anteriorly it was connected with the stomodaeum (Fig. 95) and posteriorly the end-gut was indicated (Fig. 97).

General Remarks.

One cannot view such a remarkable and unusual series of phenomena as accompanies the transformation of the young larva into the glochidium without asking one's self what is the reason of it all? Why, for instance, should the thread-gland be formed so far from its definitive position? The most natural explanation is that the primitive function of the organ in question has changed, and that a new position seemed more favorable for the discharge of the new function. On such an hypothesis there is nothing wonderful in such phylogenetic changes of position being repeated in ontogeny. We can easily apply this to the explanation of the displacements of the thread-gland. In its definitive position practically in the centre of the ventral surface, it manifestly occupies the best position for the discharge of its present function, which, as we have seen, is probably to assist the glochidium in attaining parasitic attachment to its host. For if the thread becomes attached, for instance to a fish's fin, the larva is pulled on to the fin ventral surface down; when the muscle contracts the

hooks are forced into the tissue of the fin. Now were the original position of the thread-gland to be retained, *i.e.*, at the anterior angle of the valves of the shell, the larva would not be likely to "land" on the fin in so favorable a position for attaining a secure footing. We have seen before that the primitive function of this gland was probably excretion; the change in function, then, has brought about a corresponding change in position.

I should not attempt to apply such an explanation to the movements of the oral plate and ventral plate, but would rather explain them as caused by the necessities of precocious segregation which must often isolate organs which later are intimately related.

Two factors are responsible for the redistribution of the sensory hairs in the glochidium, *viz.*, the backward motion of the thread-gland, and the invagination of the larval mantle. I have just considered the movement of the thread-gland; now as to the larval mantle. I call this the larval mantle because all authors who have described its later history state that it does not form (or does in part only) the mantle of the adult, but degenerates, giving us the so-called "fungus-like bodies" of Braun. The larval mantle is established certainly in a very curious way, and yet I think that a little consideration will convince us that at bottom it is not much different from the mantle of other forms. Its borders are formed by the evaginated edges of the shell-gland to which the shell remains attached. Practically the same thing is true for the embryonic mantle of all Molluscan forms. The difference is simply that the cavity is so enormous in this larva; and instead of being a groove-like cavity above the foot, deeper at the (primitive) posterior end, here the cavity is so great that the embryo lies within it at one end. Schmidt has attributed this concentration of the embryonic area to the immense development of the adductor muscle. It seems to me, however, an unnecessary assumption to make, for the parts in question no doubt occupy all the space they require. We should expect the embryonic material to assume a compact form, and its position at the posterior end is the natural one. The posterior end is always the growing zone of the embryo.

But why these parts are so small is a different question. We can recognize in this the degrading influence of parasitism. It would, indeed, be strange if this mode of life, which can so profoundly influence animals which have become adapted to it, as to render it a matter of speculation in what corner of the animal kingdom to classify them, had not left a deep imprint on the organization of this larva. The effect of parasitism is to exaggerate all organs essential, and to eliminate all that are inessential, to the parasite. This is precisely what has taken place in the glochidium. The larval thread, the strong muscle, the heavy shell with its hooks, and the larval mantle, are all essential to its peculiar mode of parasitism. The foot, the mouth, the intestine, the heart, *etc., etc.*, are all inessential. The former have thus been enormously exaggerated, becoming precociously impressed on the cleavage of the ovum ; the latter have been reduced to mere rudiments, the reduction also leaving its imprint on the segmentation ; but they have not been eliminated, because they are functional organs in the adult.

This is one of the most interesting of all cases of parasitism, because we have an animal fully equipped as a larva for parasitic existence, and later leading an independent life. It shows us how far parasitism can go without eliminating the possibilities of a higher evolution. It seems strange that the parasitism should finish with the larval life ; but that it does so, and that, despite its short duration, the preparation therefor should so profoundly alter the characters of the larva, is one of the best examples of the oft-emphasized fact that natural selection deals no less with the larva than with the adult. We might search the animal kingdom through without finding a better example. What a bountiful supply of transitory organs of offense and defense has nature supplied to this larva ! and all that a passing and purely larval condition should be ensured in the greatest possible number of cases !

Another important question which suggests itself, is : Why has this brief parasitic period been intercalated in the life history of this animal ? I think that Schierholz answered the question very satisfactorily when he said that it was to avoid the injurious action of the fresh water on the delicate shell of

the young animal. As parasitism gradually became the fixed habit of the species, the adaptation to the requisite conditions became more and more perfect, until the parasitism became a necessary consequence of the structure, and an indispensable condition of development.

The parasitism of the glochidium is but one way of securing protection against the injurious effects of fresh water on delicate larvae. The same protection is assured other forms (turbellarians, *Cyclas*, gasteropods) by a foetal development, which takes place either in the body of the mother or in impervious capsules. The mode of securing this end among the Unionidae is evidently correlated with the enormous number of young produced. Not that the enormous number of the young made actual viviparous reproduction such, *e.g.*, as in the Cycladidae, impossible, and hence forced, so to speak, the species to devise another means of protection. It is more logical to hold either that the parasitic habit preceded the production of such a multitude of larvae, or else that both evolved *pari passu*. We cannot, at any rate, suppose that a species could be thus perpetuated if only a few young were produced, so precarious and uncertain is the attainment of the necessary conditions of higher development.

If we suppose the Unionidae to have been derived ultimately from a marine form, we are offered a hint as to the possible mode of evolution by the present condition of *Dreissena*, which is evidently undergoing a similar change of habitat. The development of this form is a metamorphosis with a free-swimming larva, which hardly differs from the marine larvae of the same class. It is easy to picture two or three possible courses of evolution open if it becomes completely adapted to life in fresh water (in which case, as all our experience tells us, the larva would be lost). In the one case it might become purely viviparous like *Cyclas*, and produce but few young; or the ova might be deposited in impervious capsules (as, *e.g.*, pulmonates); or, again, the larva might in some way become adapted to parasitism, with consequent protection to its delicate structure. It is practically certain that in this last event the larva would change in two ways: First, so as to make it an

efficient parasite ; and second, so as to be protected, as far as possible, during its brief but necessary contact with the fresh water. To ensure the continuance of the species an enormous number of young would have to be produced. With the aid of some such a hypothesis the curious ontogeny of the Unionidae becomes more comprehensible.

(c) *Axial Relations.*

I have left till the last the consideration of the trochophore stage, and the axial relationships of the larva in the Unionidae, because it seemed better to have the whole course of develop-

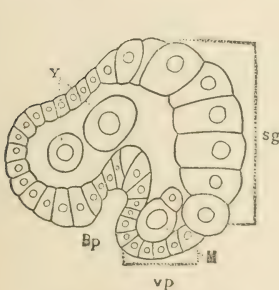


FIG. 6. — Gastrula of *Unio*.

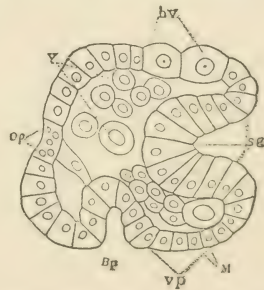


FIG. 7. — Slightly older Gastrula of *Unio*.

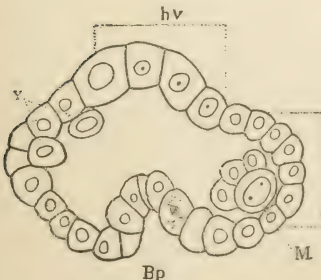


FIG. 8. — *Cyclas* (after Stauffacher).

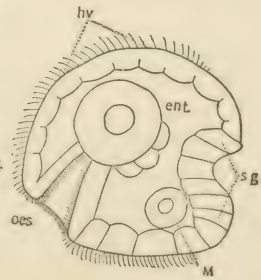


FIG. 9. — *Tereido* (after Hatschek).

Bp. Blastopore.
h.v. Head-vesicle.
o.p. Oral plate.

v.p. Ventral plate.
Ent. Entoderm.
M. Mesoblast.

oes. Oesophagus.
s.g. Shell-gland.
Y. Larval Mesoblast.

ment in mind in such considerations. The accompanying text figures (6 to 9) require but little explanation; they show that there is no real difficulty in recognizing the homologous areas in *Unio*, *Cyclas*, and *Teredo*. The latter is one of the most typical of the marine veligers; that is to say, approaches most nearly the trochophore in its structure.

Of the typical trochophore organs, the apical plate with its tuft of cilia, the praeoral and postoral rows of cilia and the head kidney are missing in *Unio*. These are of course among the most characteristic organs of the trochophore and most essential to the free life of the larva. It is these organs which always degenerate more or less subsequent to the giving up of the

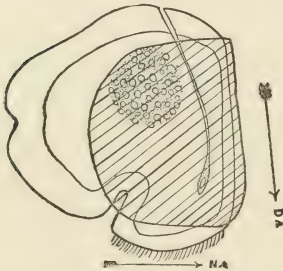


FIG. 10.—Young Larva of *Unio*.

N.A. Neural Axis. D.A. Dorsal Axis.

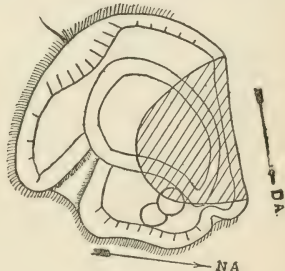


FIG. 11.—*Teredo* (after Hatschek).

[N.A. Neural Axis. D.A. Dorsal Axis.]

free life. A complete series can be traced through the various degrees of degeneracy of the organs in question to their complete absence in the Unionidae, where we can recognize only the homologous areas. The swollen cells of the head vesicle are the only remaining differentiation of the apical area which can be interpreted as rudimentary trochophore organs.

It is important to notice that in the veliger stage of all Mollusca the long axes of the shell or shell-gland and of the foot, which in the adult are parallel, are inclined at an angle of nearly 90° to one another. The figures 10, 11, and 12 in the text illustrate this in *Anodonta*, *Ostrea*, and *Teredo*. Even the most cursory examination of Gasteropod larvae will show that the same thing occurs there. This is due to the fact that the dorsal and ventral surfaces of the trunk are independently

established in these forms. The two most important factors in establishing the adult relations are the growth of the shell-gland, *i.e.*, dorsal region, and of the foot respectively. The shell-gland assumes the adult relations first owing to its early importance; the foot or neural axis is established later; this is in adaptation to its lack of function in the trochophore.

These axial shiftings have often been referred to. It will, nevertheless, be useful to review shortly the clearest accounts of them. Fol (No. 44) gives a remarkably straightforward account of the axial shiftings in the pteropods and heteropods. His statement in his pteropod paper is so concise

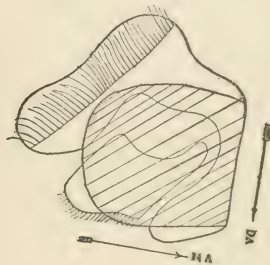


FIG. 12.—*Ostrea* (after Horst, from Korschelt and Heider).

N.A. Neural Axis. D.A. Dorsal Axis.

that it may be quoted entire (*l.c.*, p. 202). “En effet, aussitôt que les deux feuillets primitifs de l’embryon sont formés, le feuillet externe se met à croître et à s’étaler beaucoup plus rapidement d’un côté que de l’autre. Ce côté à croissance rapide répond à la région ventrale et postérieure de la larve, à celle où se trouve, on se le rappelle, la sphérule primitive protoplasmique, à celle qui donne naissance au pied et au manteau. Le tissu ectodermique qui occupait dans l’origine le pôle formatif subit de la sorte un déplacement relatif et paraît remonter le long du dos de l’embryon pour arriver enfin à la région céphalique.”

“La sphérule primitive protoplasmique” to which Fol refers is the posterior macromere. The mantle and the foot, the whole trunk in fact is traced back to the posterior macromere.

In his heteropod work Fol is, if possible, even more explicit. He shows that the region of the shell-gland is at first posterior, and that gradually it comes to lie above the mouth, *i.e.*, dorsally; at the same time the upper pole, as marked by the polar globules, is pushed around anteriorly until finally it lies at the anterior end of the body. The foot area has expanded at the same time. Thus there is a change of axis which has been accompanied by the formation of new regions, *viz.*: the shell-gland (dorsal surface), and the foot (ventral surface). Where these two regions meet posteriorly there must be a stationary area, a zone of growth. This region of growth corresponds in position to the first somatoblast of Unio.

Later authors describe a stationary area in the region indicated. Conklin (No. 40) says, "The cells of the posterior arm (of the cross) enlarge greatly and are carried forward until they lie over or even anterior to the cross-furrow, while the point at which the polar bodies are attached (the centre of the cross) is carried forward through an angle of about 90° so that it finally lies at the anterior end of the long axis of the embryo. The position which the polar bodies first occupied (immediately over the cross-furrow) coincides with the middle of the dorsal area, while the ectoderm cells which immediately surround the ectoderm pole are carried forward until they lie at the cephalic pole of the embryo. The endoderm seems to take no part in this shifting, and the ectoderm on the posterior side of the ovum is not shifted forward, but grows around in the opposite direction. There is thus a stationary point in the ectoderm on the posterior side of the ovum in front of which the ectoderm cells are shoved forward, and back of which they are shoved backward and downward. This stationary point coincides very nearly with what is later the region of the shell-gland."

This stationary point coincides also very nearly with the region of the first somatoblast. It must be a region of proliferation, anteriorly and posteriorly.

Heymons (No. 47) has witnessed the same phenomena in *Umbrella*. He says, p. 26: "Hieran sind lebhaftes Wucherungsprocesse im Ektoderm betheiligt. Dieselben schliessen sich im wesentlichen an die neuerdings auch von Conklin be-

schriebenen Erscheinungen an. Im hinteren Theil des Ektodermfeldes beginnen sich die Zellen mehrfach zu theilen, und nach vorn fortzuschieben, während gleichzeitig das durch die Richtungskörper gekennzeichnete Centrum des animalen Poles allmählich an das Vorderende gelangt. Nur der hinterste Theil des Ektodermfeldes nimmt an dieser Verschiebung keinen Antheil, sondern wuchert weiter nach hinten, d. h., nach dem vegetativen Pol hin. Die beiden Urmesodermzellen wurden dadurch gewissermassen vom Ektoderm entblösst, oder doch nur von sehr wenigen plattenförmig ausgebreiteten Ektodermzellen an ihrer dorsalen Fläche bedeckt. Letzere stellen damit die Grenze zwischen der nach vorn und der nach hinten wachsenden Partie des Ektoderms dar. Unmittelbar vor ihnen macht sich später, wenn die geschilderten Vorgänge beendet sind, weiter eine starke Vermehrung und Anhäufung von Ektodermzellen bemerkbar, die sich später in das Innere einsenken, und die Anlage der Schalendrüse bilden."

If this region of proliferation were traced further back it would probably be found that it was referable to a single cell, viz.: the first somatoblast. This is what I have done in the case of Unio. The whole ectodermal trunk region is thus traceable to the first somatoblast, the second product of the posterior macromere. The mesodermal elements are traceable to the same macromere. The whole trunk region behind the mouth can thus be traced back step by step to the posterior macromere. Dr. Whitman showed that this was true of Clepsine as far back as 1878, and Wilson has shown essentially the same thing for Nereis.

It seems to me that these facts afford a new basis for comparison of the trunk of Annelida and the postoral shell- and foot-bearing region of Mollusca. They correspond in position and in their relation to the germinal layers; it seems also that they can be traced back to identical blastomeres. I must confess that *Unio* is a form but little adapted to place this question beyond dispute. I have, however, the utmost confidence that in less highly modified forms this position will be sustained.

APPENDIX.

The appendix includes the results of some of the most important works on the cell lineage of worms and molluscs reduced to tabular form. It is inserted as a possible convenience to other workers in the same field. The system of naming the cells is the one employed in this paper, with the original designations in brackets. The necessary remarks have been made as brief as possible.

Kofoed has already published a criticism of Blochmann's work (Table I) based on the internal evidence, and I can only concur in his judgment. Referring for the evidence to Kofoed's preliminary paper (No. 49^a), I will merely note the probable errors. The trochoblasts are, according to Blochmann's derivation, $d^{2.1.2.1}$ and $b^{2.1.2.1}$ (*v. table*); but there is almost no doubt that the cells in question are a^3 and c^3 . The inner cell of the cross would be $a^{2.1.1}$ to $d^{2.1.1}$ according to Blochmann's derivation, whereas there is but little doubt that it is $a^{1.2}$ to $d^{1.2}$, thus a member of the first group of micromeres. It is, however, rather unsatisfactory to criticise a work from internal evidence alone; but until such manifest discrepancies between text and plates, as occur in Blochmann's work, are explained, it is impossible to place great reliance on them.

Rabl (Table II) has evidently been in error in the orientation of the embryos. If we are to accept his figures, the first generation of micromeres is formed leiotropically. The second generation is formed in the same way. It rotates the first generation of micromeres still further to the left. The rotation goes on, according to the figures, until finally a' is above C and c' above A . That is to say, what was on the right side is now on the left side of the embryo and *vice versa*; similarly what was anterior (of the first generation of micromeres) has become posterior and *vice versa*. It seems difficult to accept this as being really true. The first error, it seems to me, was probably in his assigning the members of the first generation of micromeres to wrong macromeres as parent cells. Thus E should not have been assigned to EJ , but to ME , *etc.*, which would make the direction of its formation dextiotropic.

It seems to me further probable that the orientation of his figure 12*a* is incorrect ; it will be noticed that the cross-furrow between E_2 and E_4 is at right angles to its direction in 11*A*. It is easier to believe that Rabl has mistaken one side of the embryo for the anterior or posterior end. This might easily be done ; for the macromeres are equal in size and the only means of orienting them is the cross-furrow, which is invisible from the apical pole. Thus the apical cross-furrow in 12*A* is probably at right angles to the vegetative as in 11*A*, and not parallel as represented. This table also shows fairly accurately the cleavage of *Limax* according to Kofoid.

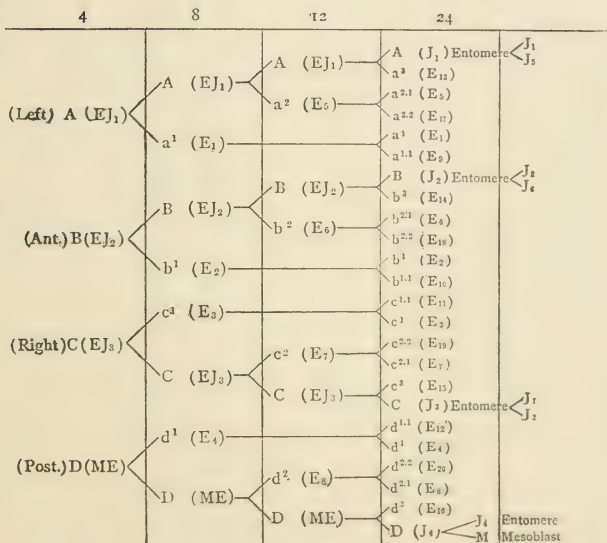
In the table of cleavages of *Umbrella* (Table III) I have not included all of the details described by Heymons after the forty-cell stage. Heymons has followed the cleavage cell by cell up to about 100 cells. The table given does not as a consequence give a correct impression of the immense detail of Heymons' work. The stages described after the 40-cell stage are : the 44-, 47-, 51-, 55-, 57-, 63-, 67-, 69-, 75-, 81-, 91-cell stages. The cleavage of the entomeres was followed far beyond these stages. It is interesting to notice the almost purely arithmetical progression in the increase in number of the cells after the four-cell stage. The disturbances in the regularity of this law are due to precocious separation of important blastomeres. *E.g.*, 24 to 25 cells due to formation of the mesoblast (*v.* table) ; 37 to 38 due to bilateral cleavage of mesoblast ; 38 to 40 another bilateral cleavage, separating excretory cells E and E' ; 55 to 57 bilateral divisions of Mesoblasts. A better illustration of Rabl's too-inclusive law could not be desired. The cleavage in *Unio* (*v.* table, p. 33) shows that precocious segregations may entirely destroy the orderly progression. The same table up to the twenty-five-cell stage at least will do equally well for *Crepidula*. Conklin has followed the cleavage very much farther, but has not yet published the details in such a way that they can be tabulated.

As Wilson has pointed out, it is probable that *v.* Wistinghausen (Table VI) has overlooked one cleavage of the posterior macromere so that the mesoblast would arise from the fourth, not from the third cleavage of this cell.

NERITINA (AFTER BLOCHMANN). TABLE I.

| 4 | 8 | 12 | 16 | 24 | 28 | 36 |
|------|----------------------------------|----------------------------------|---|----------------------------------|---|---|
| A(b) | A(b) | A(b) | A ³ (b ₃) | A ³ (b ₃) | A ^{2,1,1} (b ₂ ¹) | A ^{2,1} (b ₂) |
| | a ¹ (b ₁) | a ² (b ₂) | a ^{1,1} (b ₁ ¹) | a ² (b ₂) | a ^{2,1,2} (b ₂ ¹) | a ^{2,1,2} (b ₂ ¹) |
| B(a) | B(a) | B(a) | B ³ (a ₃) | B ³ (a ₃) | B ^{2,1,1} (a ₂ ¹) | B ^{2,1} (a ₂) |
| | b ¹ (a ₁) | b ² (a ₂) | b ^{1,1} (a ₁ ¹) | b ² (a ₂) | b ^{2,1,2} (a ₂ ¹) | b ^{2,1,2} (a ₂ ¹) |
| B(a) | B(a) | B(a) | B ³ (a ₃) | B ³ (a ₃) | B ^{2,1,1} (a ₂ ¹) | B ^{2,1} (a ₂) |
| | b ¹ (a ₁) | b ² (a ₂) | b ^{1,1} (a ₁ ¹) | b ² (a ₂) | b ^{2,1,2} (a ₂ ¹) | b ^{2,1,2} (a ₂ ¹) |
| C(d) | C(d) | C(d) | C ³ (d ₃) | C ³ (d ₃) | C ^{2,1,1} (d ₂ ¹) | C ^{2,1} (d ₂) |
| | c ¹ (d ₁) | c ² (d ₂) | c ^{1,1} (d ₁ ¹) | c ² (d ₂) | c ^{2,1,2} (d ₂ ¹) | c ^{2,1,2} (d ₂ ¹) |
| C(d) | C(d) | C(d) | C ³ (d ₃) | C ³ (d ₃) | C ^{2,1,1} (d ₂ ¹) | C ^{2,1} (d ₂) |
| | d ¹ (c ₁) | d ² (c ₂) | d ^{1,1} (c ₁ ¹) | d ² (c ₂) | d ^{2,1,2} (c ₂ ¹) | d ^{2,1,2} (c ₂ ¹) |
| D(c) | D(c) | D(c) | D ³ (c ₃) | D ³ (c ₃) | D ^{2,1,1} (c ₂ ¹) | D ^{2,1} (c ₂) |
| | d ¹ (c ₁) | d ² (c ₂) | d ^{1,1} (c ₁ ¹) | d ² (c ₂) | d ^{2,1,2} (c ₂ ¹) | d ^{2,1,2} (c ₂ ¹) |
| D(c) | D(c) | D(c) | D ³ (c ₃) | D ³ (c ₃) | D ^{2,1,1} (c ₂ ¹) | D ^{2,1} (c ₂) |
| | d ¹ (c ₁) | d ² (c ₂) | d ^{1,1} (c ₁ ¹) | d ² (c ₂) | d ^{2,1,2} (c ₂ ¹) | d ^{2,1,2} (c ₂ ¹) |

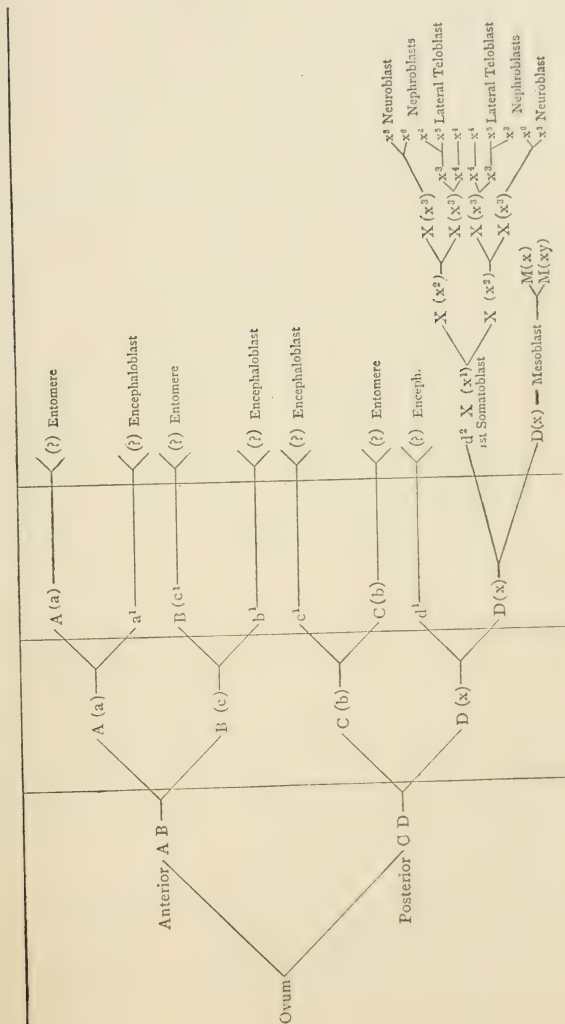
PLANORBIS (AFTER RABL). TABLE II.



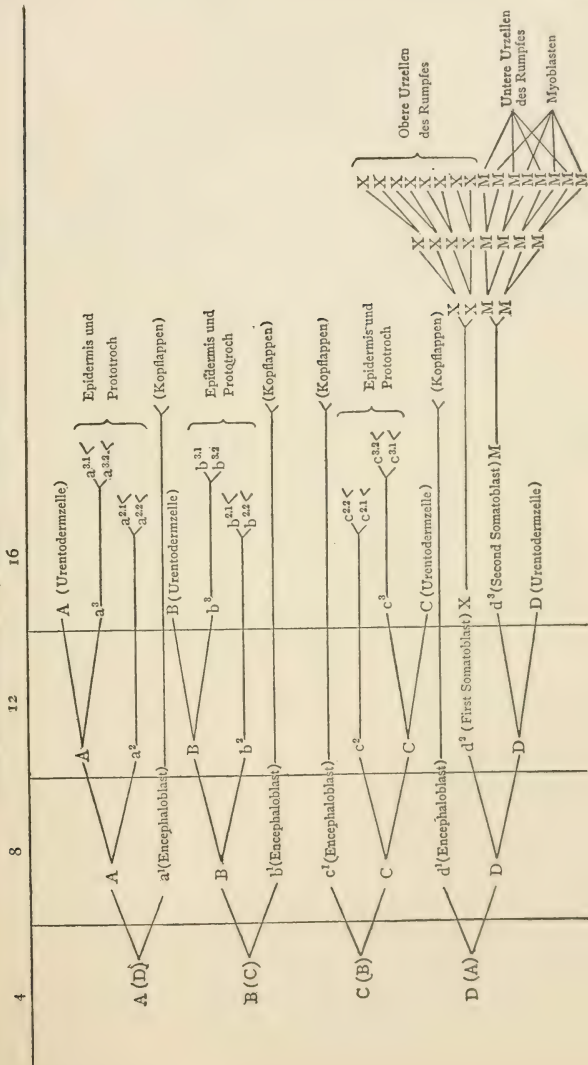
UMBRELLA (AFTER HEYMONS). TABLE III.

| | 4 | 8 | 12 | 16 | 20 | 24 | 25 | 29 | 33 | 37 | 38 | 40 |
|-------------|---|---|---------------------|--------------|----------------|--|----|---|--|----|----|---|
| | | | | A Entomere | | | | | | | | $\langle A_1 (A^1) \rangle$ |
| Left Ant. | A | A | $\langle A \rangle$ | $a^2 (a''')$ | $a^1 (a')$ | $\langle a^{21}(a_1') \rangle$ $\langle a^{22}(a'') \rangle$ | | $\langle a^{21}(a_1''') \rangle$ $\langle a^{22}(a_1'') \rangle$ | $\langle a^{21}(a_1') \rangle$ $\langle a^{22}(a_1'') \rangle$ | | | |
| | | | | | | | | | | | | |
| Right Ant. | B | B | $\langle B \rangle$ | $b^3 (b''')$ | $b^1 (b')$ | $\langle b^{21}(b_1') \rangle$ $\langle b^{22}(b'') \rangle$ | | $\langle b^{21}(b_1'') \rangle$ $\langle b^{22}(b_1'') \rangle$ | $\langle b^{21}(b_1') \rangle$ $\langle b^{22}(b_1'') \rangle$ | | | $\langle B_1 (B') \rangle$ |
| | | | | | | | | | | | | |
| Right Post. | C | C | $\langle C \rangle$ | $c^3 (c''')$ | $c^{11}(c_1')$ | $\langle c^{22}(c'') \rangle$ $\langle c^{21}(c_1'') \rangle$ | | $\langle c^{22}(c_1'') \rangle$ $\langle c^{21}(c_1'') \rangle$ | $\langle c^{22}(c_1'') \rangle$ $\langle c^{21}(c_1'') \rangle$ | | | $\langle E \text{ (Excretory-cell)} \rangle$ $\langle c''' \rangle$ |
| | | | | | | | | | | | | |
| Left Post. | D | D | $\langle D \rangle$ | $d^3 (d''')$ | $d^{11}(d_1')$ | $\langle d^{22}(d'') \rangle$ $\langle d^{21}(d_1'') \rangle$ | | $\langle d^{22}(d_1'') \rangle$ $\langle d^{21}(d_1'') \rangle$ | $\langle d^{22}(d_1'') \rangle$ $\langle d^{21}(d_1'') \rangle$ | | | $\langle E' \text{ (Excretory-cell)} \rangle$ $\langle d''' \rangle$ |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | $\langle M \rangle$ $\langle m \rangle$ $\langle M \rangle$ |
| | | | | | | | | | | | | $\langle M \rangle$ $\langle m \rangle$ $\langle M \rangle$ |

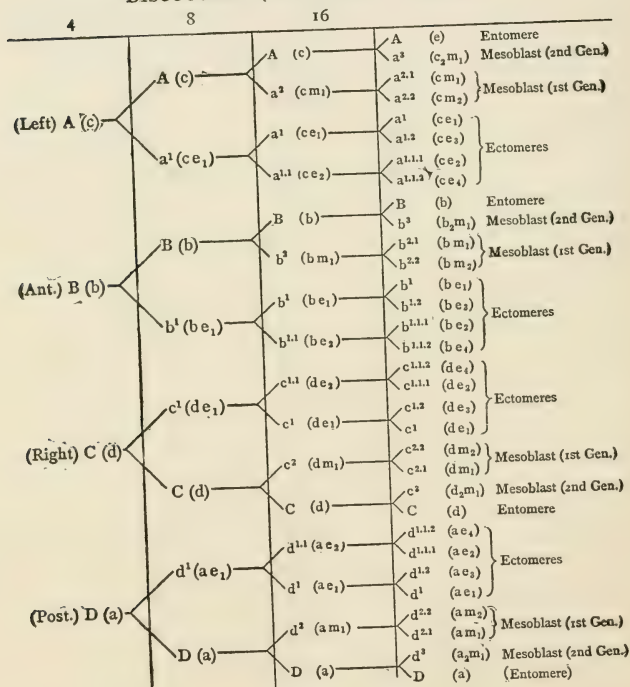
CLEPSINE (AFTER WHITMAN). TABLE IV.



NEREIS DUMERILII (AFTER V. WISTINGHAUSEN). TABLE VI.



DISCOCOELIS (AFTER LANG). TABLE VII.



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| | | | |
|---------------|---------------------------------|------------------|-----------------------------|
| <i>a.m.</i> | Adductor muscle. | <i>mes. tel.</i> | Teloblasts of mesoderm. |
| <i>ap.</i> | Apical pole. | <i>mes.</i> | Mesoderm. |
| <i>ap. n.</i> | Apical nucleus of thread-gland. | <i>mk.</i> | Micropyle. |
| <i>bp.</i> | Blastopore. | <i>o.p.</i> | Oral plate. |
| <i>c.g.</i> | Cerebral ganglia. | <i>p.t.g.</i> | Protoblast of thread-gland. |
| <i>ent.</i> | Entoderm. | <i>s.</i> | Shell. |
| <i>f.</i> | Foot rudiment. | <i>s.g.</i> | Shell-gland. |
| <i>h.v.</i> | Cells of head vesicle. | <i>s.h.</i> | Sensory hairs. |
| <i>k.a.</i> | Kidney anlage. | <i>t.g.</i> | Thread-gland. |
| <i>l.p.</i> | Lateral pits. | <i>v.p.</i> | Ventral plate. |

A Left macromere.

B Anterior macromere.

C Right macromere.

D Posterior macromere.

*a*¹, *b*¹, *c*¹, *d*¹, *a*^{1.1}, etc., First generation of ectomeres.

*a*², *b*², *c*², *d*², *a*^{2.1}, etc., Second generation of ectomeres.

*a*³, *b*³, *c*³, *d*³, etc., Third generation of ectomeres.

X = *d*² First somatoblast.

M = *d*⁴ Second somatoblast.

Y = *a*^{2.2} Larval mesoblast.

DESCRIPTION OF PLATES.

All figures were drawn with a camera lucida under a magnification of 275 diameters, except where otherwise stated. With two exceptions (Figs. 3 and 8) no attempt has been made to show the actual appearance of the segmenting ovum. A light, uniform shading has been adopted throughout for the sake of clearness. This of course does not hold for the other figures of Plates V and VI.

EXPLANATION OF PLATE I.

- FIG. 1. Egg-plates of *Unio complanata* magnified three times.
(a) The flat surface.
(b) From the side.
- FIG. 2. Part of Fig. 1 (a) more highly magnified.
- FIG. 3. Ovum within vitelline membrane; polar globules have been formed opposite to the micropyle (*m k.*).
- FIG. 4. Early two-cell stage.
- FIG. 5. Later two-cell stage in outline.
- FIG. 6. Preparatory to three-cell stage from the upper pole.
- FIG. 7. Three-cell stage from the upper pole.
- FIG. 8. Three-cell stage from the lower pole. Actual appearance of egg shown.
- FIG. 9. Early four-cell stage from the upper pole; cross-furrow small. I-I, first cleavage-furrow; II-II, second cleavage-furrow.
- FIG. 10. Later four-cell stage from the upper pole; cross-furrow large. I-I, first cleavage-furrow; II-II, second cleavage-furrow.
- FIG. 11. The four-cell stage from in front.
- FIG. 12. The five-cell stage from the upper pole. This egg is remarkable inasmuch as *A*, not *D*, is the largest of the macromeres. The lower ends of the spindles in *B* and *C* are outlined more faintly than the upper ends.
- FIG. 13. Six-cell stage from the upper pole.
- FIG. 14. Six-cell stage from in front.
- FIG. 15. Eight-cell stage from above.



EXPLANATION OF PLATE II.

FIG. 16. Eight-cell stage from the right side. Lower pole rolled up a little.

FIG. 17. Passage to nine-cell stage from behind. Formation of first somatoblast, $d^2 = X$.

FIG. 18. Anterior view of the same egg.

FIG. 19. Ten-cell stage from the right side. Formation of the second generation of ectomeres.

FIG. 20. Thirteen-cell stage from the upper pole. d^1 of the first generation of ectomeres has divided. The large cells of the second row are the members of the second generation of ectomeres.

FIG. 21. The same egg from the right side, showing the arrangement of the macromeres also.

FIG. 22. Seventeen-cell stage from the upper pole. All of the first generation of ectomeres have divided. x^1 is the seventeenth cell.

FIG. 23. The same egg from the vegetative pole. The spindle in D is for the separation of d^3 to the left.

FIG. 24. Eighteen-cell stage from the upper pole and partly from in front. Spindles in a^2 , b^2 , c^2 , and d^2 (cf. Fig. 25).

FIG. 25. The same egg from the left side. The spindle in a^2 separates $a^{2,2}$ — the larval mesoblast.

FIG. 26. Eighteen-cell stage directly from above.

FIG. 27. The same egg from the lower pole.

16.



20.



21.



22.



23.



24.



25.



26.



27.



EXPLANATION OF PLATE III.

FIG. 28. Twenty-two-cell stage from the upper pole ; rolled somewhat to the left.

FIG. 29. The same egg from the left side. Larval mesoblast—*Y*—partly overgrown by x^2 and d^3 (*cf.* Pl. II, Fig. 25).

FIG. 30. Slightly later stage from the upper pole. Second division of d^1 .

FIG. 31. Formation of x^3 . Upper pole.

FIG. 32. Same egg. Lower pole. Formation of third generation of ectomeres.

FIG. 33. Upper pole. Formation of x^3 .

FIG. 34. Lower pole. Formation of third generation of ectomeres.

FIGS. 35-38. Four views of the same egg after the formation of x^3 and the third generation of ectomeres. Twenty-seven-cell stage.

FIG. 35. Upper pole.

FIG. 36. Anterior view; in part from above.

FIG. 37. Direct view from in front.

FIG. 38. Lower pole.

FIG. 39. The next stage from below. The indicated cleavage of *D* completes the separation of the germinal layers. x^4 in process of formation.



EXPLANATION OF PLATE IV.

- FIG. 40. Thirty-one-cell stage. Lower pole.
- FIG. 41. Lower pole. Division of $c^{2.1}$.
- FIG. 42. Lower pole. Division of $b^{2.1}$, $b^{2.2}$, $c^{2.2}$ and d^3 . Thirty-seven-cell stage.
- FIG. 43. The same stage from in front.
- FIG. 44. Equal division of X . First bilateral cleavage. Notice relations of x^1 , x^2 , x^3 , and x^4 to X . View from behind.
- FIG. 45. Lower pole at the time of the bilateral division of M .
- FIG. 46. View from behind. Formation of x^5 .
- FIG. 47. Same stage from the right side.
- FIG. 48. Upper pole of stage of Fig. 44. Division of a^1 and $a^{2.1}$.
- FIG. 49. Upper pole. Division of c^1 and a^1 .
- FIG. 50. Upper pole. Sixteen cells of the first generation of ectomeres.
- FIG. 51. Slightly later stage. Division of $d^{1.2}$. Stage of Fig. 45. Fifty cells.

EXPLANATION OF PLATE V.

FIG. 59. Left side of embryo of over fifty cells, showing inclusion of *Y* and formation of *y*².

FIG. 60. Superficial budding of the mesoblasts.

FIG. 61. Horizontal optical section of stage with four cells of the shell-gland. The larval mesoblast — *Y* — is passing into the segmentation cavity. Notice the asymmetry of the primary mesoblasts — *M*.

FIG. 62. Transverse section (actual) of a later stage, showing bilateral cleavage of *Y*.

FIG. 63. Optical section in the same plane as Fig. 61. Divisions of *M* and *Y*.

FIG. 64. View of gastrula from dorsal surface. The large cells are the rudiment of the shell-gland.

FIG. 65. Gastrula from lower pole. Entoderm in sepia.

FIGS. 66–67. Successive sagittal sections of the stage of Figs. 64, 65, and 72. Fig. 66 passes a little to one side of the middle line; Fig. 67 directly in the median line. See line of section in Fig. 65.

FIG. 68. Section of same stage through shell-gland and blastopore. See line of section in Fig. 65.

FIG. 69. Median sagittal section after the invagination of the shell-gland.

FIG. 70. Horizontal section of the same stage as Fig. 69.

FIG. 71. The six cells from the region of the thread-gland. The central cell is the protoblast of the gland. From the same stage as Fig. 69.

FIG. 72. Gastrula from the side.

FIG. 73. Median sagittal section of larva considerably younger than stage of Fig. 79.

FIG. 74. Part of Fig. 73 more highly magnified (Leitz Hom. oel-immers., $\frac{1}{12}$). Compare apical nucleus of thread-gland with nuclei on either side of aperture; compare, also, with Fig. 71.

FIGS. 75–78. Transverse sections from before-backward of the stage of Fig. 73. For region of sections, *v.* Fig. 73.

FIG. 79. Young larva of *Unio* from the right side. *Cf.* apical nucleus with *ap. n.* of Figs. 73 and 74. *Extremely fine* cilia of ventral plate not represented.

FIG. 80. The same from in front.

FIG. 81. Arrangement of nuclei ($\times 500$) around opening of thread-gland of Fig. 80. (*Cp.* Fig. 71.)



EXPLANATION OF PLATE VI.

FIG. 82. Embryo slightly older than Fig. 79; seen from the ventral surface. Invagination of larval mantle begun.

FIG. 83. Obliquely sagittal section of stage of Fig. 82; *v.p.*, ventral plate.

FIG. 84. Median sagittal section of same stage.

FIG. 85. Ventral half of transverse section through stage of Fig. 82. Shell not drawn in.

FIG. 86. Horizontal section through ventral wall of same stage.

FIG. 87. Section slightly dorsal to Fig. 86 (*v.* Fig. 84 for plane of section).

FIG. 88. Median sagittal section of the young glochidium of *Unio complanata*.

FIG. 89. Transverse section of the same stage, passing through the intestine.

FIG. 90. Same series; three sections ($7\frac{1}{2} \mu$) in front.

FIG. 91. Horizontal section of same stage, showing opening of thread-gland into mantle cavity.

FIG. 92. February Glochidium of *Anodonta*, anterior view; only a mere fraction of thread drawn.

FIG. 93. Ventral view of same; shell gaping.

FIGS. 94-97. Four transverse sections from stage of Fig. 93. Sections asymmetrical, passing further forward on left side.

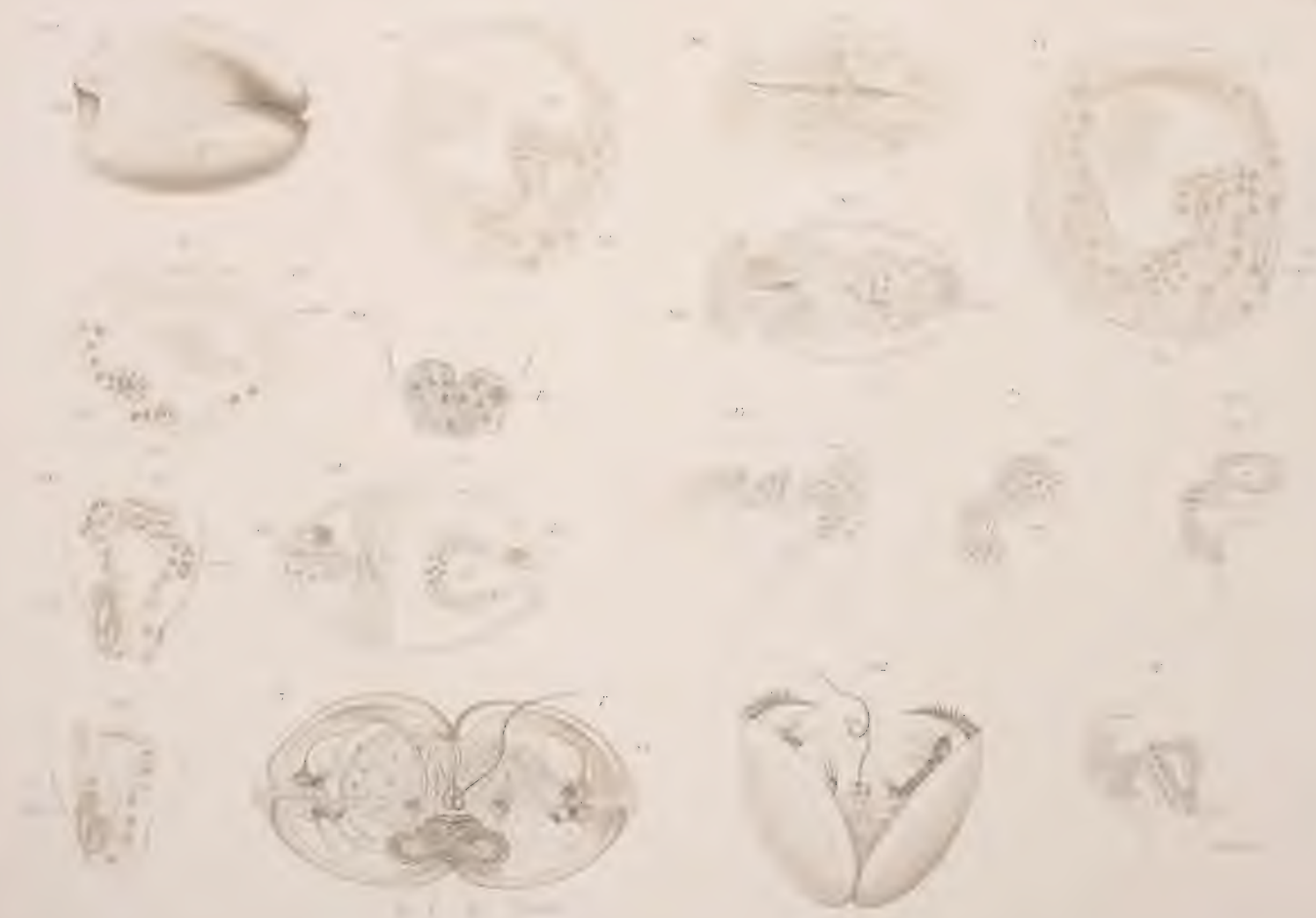
FIG. 94. Through cerebral ganglia.

FIG. 95. Three sections posterior to Fig. 94; *oes.*, rudiment of oesophagus.

FIG. 96. Five sections posterior to Fig. 94.

FIG. 97. Six sections posterior to Fig. 94.

(Sections 7.5μ thick.)



THE CRANIAL NERVES OF AMPHIBIA.

A CONTRIBUTION TO THE MORPHOLOGY OF THE VERTEBRATE NERVOUS SYSTEM.

OLIVER S. STRONG.

COLUMBIA COLLEGE.

ANALYSIS OF CONTENTS.

| | PAGE |
|---|------|
| INTRODUCTION | 101 |
| I. TECHNIQUE | 103 |
| II. DETAILED DESCRIPTION OF THE NERVES AND THEIR COMPONENTS..... | 106 |
| 1. <i>Trigeminus</i> | 107 |
| 2. <i>Facialis and Acusticus</i> | 112 |
| 3. <i>Glossopharyngeus and Vagus</i> | 135 |
| 4. <i>Sympathetic</i> | 150 |
| 5. <i>Recapitulation of nerve components</i> | 151 |
| III. COMPARATIVE MORPHOLOGY OF COMPONENTS..... | 153 |
| 1. <i>General cutaneous</i> | 153 |
| 2. <i>Special cutaneous or lateral line</i> | 158 |
| (a) <i>Amphibia</i> | 158 |
| (b) <i>Fishes</i> | 160 |
| (c) <i>Résumé</i> | 178 |
| 3. <i>Fasciculus Communis</i> | 180 |
| (a) <i>Amphibia</i> | 180 |
| (b) <i>Higher Vertebrates</i> | 185 |
| (c) <i>Fishes</i> | 188 |
| 4. <i>Motor</i> | 197 |
| 5. <i>Comparison with Cyclostomes</i> | 198 |
| IV. GENERAL CONSIDERATIONS | 201 |
| 1. <i>Relation of Cranial and Spinal Nerves</i> | 201 |
| 2. <i>Relations of the Pre- and Post-Auditory Nerves</i> | 206 |
| 3. <i>Bearings upon the Classification and Segmentation of the Nerves</i> | 208 |
| V. APPENDIX ON TECHNIQUE | 211 |

INTRODUCTION.

THIS research may be regarded as, in a manner, a continuation of Professor Osborn's work on the Amphibian brain. In the introduction to his paper, "A Contribution to the Internal Structure of the Amphibian Brain" (JOURN. OF MORPH., Vol.

II, No. 1, July, 1888), occur the following sentences: "Much remains to be done in respect to the *peripheral distribution of the component parts of the several cranial nerves*. Only after this has been thoroughly worked out can we certainly determine the homologies of the cranial nerves and their segmental relations in the Amphibia. The present results go far enough to show that the determination of definite nuclei corresponding to definite peripheral sensory and motor areas is well within the range of possibility. In fact, the provisional character which I have given to some of the conclusions here reached is chiefly due to the close connection between several of the cranial nerves at, or close to, their exit, which makes it necessary to follow each component bundle in continuous sections peripherad to a point where their further distribution can be traced macroscopically. This, I believe, is possible with several of the nerves, but has not as yet been successfully accomplished."

To supply some of these deficiencies was the immediate aim of this investigation, and, I think, some of the obscure points mentioned by Osborn in his paper are satisfactorily cleared up. While very much remains to be done on the forms here investigated, yet a firmer basis has been acquired on which to make comparisons with the cranial nerves in other forms and to draw conclusions as to their nature.

In the opinion of the writer much of the embryological work upon the cranial nerves has been very defective and even misleading, owing to the fact that their adult distribution and function had not first been accurately worked out. I would venture to predict that more light can and will be thrown upon the nature of the cranial nerves in the immediate future by means of anatomical and histological than by purely embryological research.

This paper is by no means a monographical account of the cranial nerves of Amphibia, but will be confined principally to the V, VII, IX, and X nerves. Other nerves will be included in the description as they come into connection with these.

The types upon which the bulk of the work has been done and upon which the description is based are several common

species of *Rana* in the late larval stages of development. Other Amphibia, especially the *Amblystoma* larvae, are brought in incidentally as throwing light upon certain points.

This research was begun at Princeton in 1889 at the suggestion and under the direction of Prof. Henry F. Osborn. A summary of the results first obtained appeared in the *Zoologischer Anzeiger*, Nr. 348, 1890 (61). At the Lake Laboratory, established by Mr. Allis at Milwaukee, I was enabled to make some additions to these results, which appeared in the *Anatomischer Anzeiger*, Nr. 15, 1892 (62). The final publication has been delayed, owing partly to other duties and partly to further observations made while Fellow in Biology at Columbia College, and also during the past year.

I wish to express here my deep indebtedness to Professor Osborn for many valuable suggestions and for his assistance in many ways in this research. For the execution of the majority of the drawings I am indebted to Dr. Arnold Graf, to whose skill they bear witness.

I. TECHNIQUE.

A considerable part of the investigation was upon tadpoles fixed in Perenyi's fluid, stained *in toto* in carmine, imbedded in paraffin and cut in serial sections. These were mounted in order upon large glass slides, strips of thin mica being used as covers. The carmine staining is not to be highly recommended, as carmine solutions do not stain very differentially, and would probably be even more unsuitable with forms where the tissues are more compact than in the tadpole.

The *osmium-bichromate* mixture used for hardening in the Golgi method gives in itself a very good stain for the medullated fibres of the peripheral nerves, and sections prepared by means of this method were thus doubly useful.

The Golgi Method.—For the terminations of the nerves and for demonstrating certain tracts consisting of many non-medullated fibres, the Golgi method proved invaluable. It may be well to describe here in detail the precise procedure which I have found useful and convenient.

Preliminary to hardening, the manner of cutting the tadpole is a matter of some importance. The special region should be opened up to some extent in order to insure a speedy and complete penetration of the osmium-bichromate and the silver nitrate solutions, as neither of them have much penetrating power. On the other hand, by not cutting into too small pieces the precipitate formed by the silver is not deposited so extensively on all the surfaces, and many beautiful pictures can thus be obtained not marred by its presence. In investigating the head region I have found it best to cut the animal transversely into three pieces, one cut being made a short distance caudad of the auditory capsule and Vagus, and the other passing just through the anterior surfaces of the eyeballs. It is, perhaps, best to allow the animal to remain in the fixing fluid half an hour or so first, to acquire a better consistency for this cutting.

With respect to the *osmium-bichromate* mixture, a considerable latitude in the proportions may be taken and good impregnations obtained; the same holds good as to the strength of the *silver nitrate* solution. For example, good preparations were obtained from a tadpole impregnated as follows: Pot. bich. sat. sol. 140 cc. + osmic 1% 10 cc., 21 days, silver nitrate 10%, 3 days. The following, however, are the two solutions chiefly used, the first one (1) being after Berkley (10) and the second (2) very much like that recommended by Ramón y Cajal: (1) Pot. bich. 5% 84 cc. + osmic 2% 16 cc., and (2) Pot. bich. 3½% 4 vols. + osmic 1% 1 vol. I cannot say that I have found much choice between the two.

As seen above, impregnations may be obtained after hardening for 21 days in a solution containing *less* osmic. On the other hand good impregnations of the more superficial parts were obtained in one case as follows: Pot. bich. 3½% 4 vols. + osmic 1% 1 vol., 22 hours, during the first 11 hours in a solution which had been previously used, silver nitrate ¾%, 2½ days. This shows that here again there may be a considerable range within which good results are obtainable. There is a certain hardening period, however, which is much the most favorable; this is 2 to 5 days, and probably about 3 days will usually give the best results throughout the pieces.

Lithium Bichromate Modification for Adult Brains.—I have made a number of attempts to dispense with osmic acid in the fixing fluid, but without success for the tadpole. A modification was discovered, however, which gives some very fine impregnations in the adult mammalian cerebrum and cerebellum, *dispensing with osmic acid and yet requiring only a few days for the process.* It consists simply in the use of *lithium bichromate* instead of potassium bichromate. This salt is very soluble in water and makes a solution somewhat darker than the potassium bichromate, which it resembles. It hardens very much more rapidly than the latter, so that small pieces of brain placed in a 3% solution require only a day or two instead of 20 to 30 days to reach the condition favorable for impregnation. This favorable condition, however, is passed through very quickly. I have not had opportunity, as yet, to test this method thoroughly, but have obtained splendid pictures of the Purkinje and pyramid cells, and also in the fowl's brain of the granule cells, showing the T-shaped division of their axis cylinders in the molecular layer of the cerebellum.

Sodium Sulphate Modification for Increased Penetration.—One of the defects of the Golgi method, especially of the rapid method, seems to lie in the poor penetration of the silver nitrate, resulting in irregular and defective impregnations. It occurred to me that by combining the silver solution with some salt that would be indifferent chemically in the reduction of the silver, but would facilitate its penetration, the results might be improved. Two salts were tried, namely, *sodium sulphate* and zinc sulphate. Both seemed to tend to produce the desired effect, for some of the best and most thorough impregnations were obtained from specimens treated especially by the former. The mixture of silver nitrate and the sodium sulphate was made in varying proportions. In two of the most successful impregnations the following were the solutions into which the objects were brought from the osmium-bichromate mixture: (1) Sodium sulphate 6% 1 vol. + silver nitrate 4% 1 vol.; (2) sodium sulphate 8% 1 vol. + silver nitrate 1% 1 vol. A precipitate is formed in mixing these, and it would be advisable to so adjust the proportions as to prevent this.

Equal volumes of 1% solution of each avoided this, and also gave some good results. *Zinc sulphate* seems to act similarly to the sodium salt and can be mixed with the silver in larger proportions without producing a precipitate.

It must not be understood that this latter modification gives ideal results. In some cases it seems to be an improvement, but further experience is necessary to ascertain its precise value. In the tadpole it has yielded especially good preparations of the nerve terminations in the heart (Pl. IX, Fig. 14). The specimens should be placed in pure silver nitrate a while previous to placing them in alcohol, in order to wash out the sulphate, otherwise the alcohol will precipitate the latter in the tissues. The specimens are left in the dark while in the osmium-bichromate and in the silver bath.

Preservation.—If, for any reason, specimens cannot be cut and mounted immediately after impregnation, they can be best preserved in the silver bath. Specimens will often keep thus for months, but there is considerable risk of deterioration. This latter is probably due, as von Lenhossék suggests (37), to a slow precipitation of the silver in solution, so that in time the specimen is left simply in water which, in turn, bleaches out the stain. Another cause of deterioration appears to be a gradual darkening of the whole tissue. It is obvious from this that if it is necessary to keep the specimens some time before cutting, two precautions should be taken, (a) the specimen should be kept in a liberal supply of the silver solution of full strength, and (b) it should be kept strictly in the dark in order to prevent, as far as possible, a gradual secondary reduction of the silver.

The double and triple impregnation, as recommended by Cajal (13), was tried with good results. The exact details of procedure in technique are placed in an appendix.

II. DETAILED DESCRIPTION OF THE NERVES AND THEIR COMPONENTS.

The most direct treatment seemed to be, first, to examine each nerve in detail, with a view to ascertaining its components, each section concluding with a table summarizing the

results. The term '*component*' is not necessarily synonymous with '*root*,' for often *two or more* components different in fibre structure, internal origin, distribution, and function are given off as *one* root, and *vice versa*, different roots may be composed of similar components. 'Root' has reference to the number of separate bundles by which a nerve issues from the central nervous system — while by '*component*' we refer to bundles qualitatively different. In some cases, of course, the two are identical.

A résumé of this part treats the nerves collectively as regards their components.

Second, each of the main components thus determined will be further considered as representing a system, and used as the basis of homologizing the cranial nerves of the Amphibia with those of other orders.

The chart (Pl. XII, A) was reconstructed from a series of transverse sections through the tadpole by plotting out the nerves, etc., upon a sheet of paper ruled in squares, the relation between the thickness of the sections and the magnification having been first ascertained. For conciseness and precision the numbers of the sections are used in the text to indicate distances measured along the longitudinal axis. As the sections were 10μ thick, these numbers divided by 100 will give the actual distances in millimeters and decimals of a millimeter. Each interval in the scale along the sides of the chart equals 10 sections ($= 100\mu$), and the numbering corresponds to that used in the text. This correspondence, however, is not always exact, owing to slight changes made in the chart in its preparation.

1. *The Trigemini.*

The *Trigemini* emerges from the side of the medulla .10 mm. cephalad of the VII + VIII roots (866-848). Its exit takes place principally just anterior to the entrance of the posterior branch of the VIII into the auditory capsule. It proceeds obliquely cephalad occupying, together with the other nerves described below, a position in the cranium immediately inside the ventral portion of the auditory capsule. The bulk

of its fibres are rather small, but with a number of medium-sized fibres among them, and a still smaller number of large fibres. The ascending tract of the V contains a few scattered large fibres, and the ventral root of the V. (V minor) is coarse fibred.

After proceeding cephalad .7 mm. it becomes ganglionated, .36 mm. further on its Gasserian ganglion begins to divide into a dorsal and a ventral part, the ventral part at the same time passing through the floor of the cranial cavity and consequently lying in the roof of the mouth. Here this ventral portion has lost its ganglion cells and becomes the *Ramus ophthalmicus trigemini*. This partial division of the anterior extremity of the Gasserian ganglion is the only sign of separation between the ganglia of the Rr. ophthalmicus and maxillo-mandibularis trigemini respectively. The R. ophthalmicus continues cephalad and gradually dorsad, thus entering the orbital cavity. .3 mm. cephalad of its separation from the rest of the V, it comes into connection with the III nerve, which divides on its inner side, one part of the III passing up around it, and the other bending forwards beneath it.

During the remainder of its course the R. ophthalmicus gives off several branches to the skin, which need not be described more in detail here. When it breaks up in the anterior extremity of the head one branch is given off (170), which bends down, pierces a layer of fibrous cartilage which separates the skin from the oral subepithelial layers and divides. One division, proceeding caudad, is continuous with a branch of the R. palatinus VII. The significance of this connection will be discussed in the description of the latter. The other division of the R. ophthalmicus, possibly together with some fibres from the R. palatinus VII, proceeds cephalad a short distance, and breaks up into a rich plexus, terminating in the epithelium of the roof of the anterior extremity of the oral cavity. This plexus and its terminations are figured in Pl. VII, Fig. 3, where the plane of the section enables one to obtain a view of its mode of branching and termination.

The fibres of the R. ophthalmicus V are rather small, but of variable size, and with, perhaps, a dozen and a half coarse

fibres scattered among them. As the different branches separate from the main trunk they usually draw off several of these larger fibres. The latter have apparently no special significance as far as their peripheral distribution is concerned. They seem to have the same cutaneous terminations as the smaller fibres. Whether a more exhaustive study of them would reveal histological differences in their ultimate terminations, I do not know. The presence of these large fibres in cutaneous branches is readily accounted for by their presence also in the ascending tract of the Trigemini, in which they can be traced caudad to the posterior columns of the cord.

The other and dorsal division of the V .46 mm. cephalad of its separation from the R. ophthalmicus separates into the *Rr. maxillaris* and *mandibularis*. The last ganglion cells disappear .7 mm. cephalad of the first, thus making .7 mm. the length of the Gasserian ganglion.

Slightly cephalad of the subdivision into the *Rr. maxillaris* and *mandibularis* the motor branches of the Trigemini to *Mm. pterygoideus* and *temporalis* are given off. These branches all arise together from the same point on the ventral side of the *R. mandibularis*. Some distance cephalad of this, and in about the same transverse plane as the posterior nares, the *R. mandibularis* gives off branches innervating the *M. masseter*. Still further along it bends mesad and gives off the musculo-cutaneous branch to the *M. submaxillaris* (*mylohyoideus anterior*) and the skin beneath it. As it finally breaks up it innervates the *Mm. submentalis* and *mandibulo-labialis* of Schulze (54).

The cutaneous branches which compose the bulk of the *R. mandibularis* need not be described here. The general manner of termination of cutaneous nerves will be touched upon below. The character of the fibres of the *R. mandibularis*, as well as of the fibres of the *R. maxillaris*, is similar to that of the fibres of the *R. ophthalmicus*, and what has been said of the latter applies to them also.

Besides its motor and cutaneous branches, the *R. mandibularis* innervates a part of the epithelium of the mouth. At 200 a twig is detached which proceeds mesad to the mouth at

150±, about in the same transverse plane as the termination of the R. mandibularis VII, and innervates the epithelium lining the under side of a lateral diverticulum of the oral cavity.

At the terminal portion of the R. mandibularis one branch also proceeds dorsad and then along beneath the epithelium of the labial cartilage (170±). It forms here a dense plexus in the subepithelial connective tissue layer and apparently in close apposition to the cartilage. The appearance of this plexus is difficult to reproduce, but Pl. VII, Figs. 5, 6, and 7, will give some idea of its character. The stain is not so black as that of the other nerve fibres, and the fibres of the plexus certainly appear to fuse with each other, forming a true network. This appearance is not so apparent in sections in which the stain is less complete—or in which the plexus is more diffuse,—and may possibly be due to an excessive precipitation of the silver. I am not inclined, however, in view of the appearances presented to accept this explanation. In this plexus are numerous varicosities and many free endings terminating in small knobs similar to the varicosities.

From this plexus arise at right angles innumerable twigs which break up into arborisations in the epithelium surrounding the cartilage. The fibres of this plexus do not anastomose but simply interlace. A good idea of their appearance is given in Pl. VII, Fig. 5. The thicker fibres from which they arise represent the perichondral plexus mentioned above and which Figs. 6 and 7 represent in horizontal section.

It is noticeable that none of these fibres, or very few, penetrate more than about two-thirds of the thickness of the epithelium. I think it is not unlikely that the explanation of this lies in the character of the epithelium. The outer layers of the latter consist of more flattened cells which are probably partly cornified.

It is difficult to see exactly what the significance of the plexus or network closely enveloping the cartilage is. It corresponds to the basal plexuses lying in, or under, other epidermal and epithelial structures and from which the terminal fibres arise. Here, however, its unusually compact character, its close apposition to the cartilage and the great number of

endings it contains would seem to indicate, in addition, some special function.

With one exception the branches of the *R. maxillaris* need not be described further. This one is given off at about 310, passes cephalad and mesad and here subdivides. One subdivision is continuous with a branch of the *R. palatinus* VII while the other, together, apparently, with a portion of the *R. palatinus*, proceeds cephalad along the side of the oral cavity supplying its epithelium with fibres and finally breaking up at its extreme anterior end (Pl. VII, Fig. 2).

Besides these branches of the Trigemini there are a number of others which have been but little noticed and yet, though small, seem to be of some morphological importance. There are usually three of these and all are given off in about the same transverse plane, and about .3 mm. caudad of the division of the V into its maxillary and mandibular branches. Two of these arise from the inner side of the V, — from the inner side of the anterior extremity of the Gasserian ganglion, — and one can be traced caudad along its inner side almost to the point of separation of the *R. ophthalmicus*. The third and largest branch arises apparently from the outer side of the V but its fibres can be seen passing mesad across the dorsal side of the V, so that they ultimately originate from about the same point as do the other two. These *accessory* branches seem to derive their fibres, in part at least, from the few large ganglion cells in the dorsal and mesal side of the trunk of the V, constituting the apex of the Gasserian ganglion.

These branches, like the other branches of the V, consist principally of small fibres with a few large ones among them. They fuse temporarily with certain branches of the VII, as will be described below.

It will not be necessary to describe the branches of the V further as no departures of importance from, or additions to, the usual descriptions have been noted.

The cutaneous terminations of the fibres of the various branches of the Trigemini (Pl. VII, Figs. 1 and 4,) present no especial differences among themselves. Their modes of branching and courses differ, however, in different tadpoles, and these

differences evidently depend upon differences in the structure of the skin, in the thickness of its different layers and the form and arrangement of the epidermal cells. The branches break up in the deepest layer of the cutis, bend at right angles and form a heavy, coarse plexus of nerve fibres extending parallel to the surface. From this plexus one or several fibres pass vertically through the middle layer of the cutis and in the superficial layer break up into terminal arborisations, the fibrils of which pass into the epidermis, there to ramify still further. The course and configuration of these ramifications is correlated in a general way with the shape of the epidermal cells. In passing through the middle layer of the cutis the vertical fibres give off branches at right angles which course along between the dense parallel strands of connective tissue which constitute this layer. The endings in the different layers appear to be always free and intercellular. The precipitate formed on the surface often interferes with following them to their final terminations.

Immediately beneath the middle layer of the cutis, and still more abundantly in the superficial layer immediately beneath the epidermis, a number of dark bodies are present (Fig. 4, *x*), usually with a smooth oval outline, and are, apparently, a species of pigment cell. While the nerve fibres now and then lie close to these, there is no connection between the two. I have not found any cells, such as those described by Eberth and Bunge (16) in the foot of the frog. While negative evidence, especially with Golgi preparations, is far from conclusive, yet I think, judging even from their own figures, their results are open to the criticisms made upon them by Van Gehuchten (27).

2. *The Facialis and Acusticus (Auditory).*

The *Facialis* and *Acusticus* present even greater difficulties in the tadpole than in the Urodela. In the latter the exits of the *Acusticus*, and what is here called the *dorsal VII*, are quite distinct. The latter is considerably reduced in the tadpole and *in proportion as it is reduced the Acusticus is increased*, the exit of the *VIII* being extended so much dorsad that it and

the dorsal VII emerge from the medulla together and only become separated later in their course. An interesting question here arises. Does the Acusticus in its extension dorsad appropriate a portion of the dorsal VII? This could best be determined by a careful study of these nerves and their internal origin through the stages of transformation into the frog. If such a transference takes place it would lead to the remarkable result that the Acusticus of the Urodela is not strictly homologous with that of Anura. It should also then be determined what structures in the ear receive this increased nerve supply. Some further aspects of this question will be dealt with later.

The exit of the VII + VIII (899-876) occupies a large portion of the side of the medulla. In the most caudad part of this exit there may be seen fibres leaving the medulla which, when traced internally, curve ventrad, and evidently have an origin much inferior to that of the bulk of the VIII. This root has been observed by Stieda (59) and others, and is spoken of in Ecker and Wiedersheim's *Anatomy of the Frog*. (17) as derived from the motor trigeminal nucleus. Osborn (45), however, has demonstrated that a similar ventral rootlet in *Cryptobranchus* is derived directly from the posterior longitudinal fasciculus. My sections, being of smaller brains, are inadequate for the settlement of the derivation of this root in the tadpole. I think it probable that some of the fibres originate from a portion of the trigeminal motor nucleus, and possibly others may come from the posterior longitudinal fasciculus. I have so indicated them in the chart.

These fibres, as they emerge from the medulla, form a bundle close under the large root of the Acusticus. This bundle was termed, in the abstract in the *Zoologischer Anzeiger* (61), the "*ventral root of the ventral VII*" or "*VII ab.*"

Slightly cephalad of this, and between it and the Acusticus, another root can be distinguished, composed of fine fibres, which are derived from a bundle representing the *fasciculus communis* of Osborn (Pl. XI, Fig. 39). This was designated "*VII aa*" in the *Anzeiger* abstract. It fuses with the first root and these two ventral roots of the VII were, for con-

venience, together denominated the "*ventral VII*," a name, as seen immediately below, not applicable to some other forms. There is one remarkable peculiarity in connection with this root in the larvae and adults of *Anura* as contrasted with *Urodela*. In the latter, as described by Osborn in *Cryptobranchus*, its exit is just dorsal to the *Acusticus*, while in the former, as described above, its exit is just ventral to the *Acusticus*. This transference is probably connected with the change in position of the *Acusticus* as noted above.

These two roots fuse with the ventral side of the root of the *VIII* so closely that tracing them in the tadpole is a matter of some difficulty.

The *Acusticus* shows the division into dorsal and ventral roots described by Köppen (35). In the caudal part of the former the fibres are smaller than those of the latter, which are of varying sizes but contain some very large fibres. The fibres of the dorsal root proceed obliquely ectad and ventrad, and immediately pass within the auditory capsule and enter the posterior portion of the auditory ganglion, which is composed of small ganglion cells. The dorsal part of the ganglion further cephalad, however, becomes composed of large ganglion cells which supply the coarser fibres of the auditory branches. The posterior branch of the *VIII* consists of both coarse and smaller fibres, as does the anterior, but the latter branch seems to contain a larger proportion of coarse fibres. The larger part of the dorsal root of the *VIII* seems to supply the posterior auditory branch and the larger part of the ventral root the anterior branch.

The most dorsal fibres, belonging apparently to the cephalic portion of the *VIII* at its exit, separate from the *VIII*. They form what was termed in the abstract the "*dorsal VII*" or "*VII b*." This root can be seen to arise in part from fibres running longitudinally in the dorsal part of the medulla at this place. According to Osborn's observations on *Cryptobranchus* (45) they ultimately arise from nuclei in this part of the medulla. It may be remarked here, however, that the connection between "sensory" nuclei in the central nervous system, such as those mentioned by Osborn, and the roots of

ganglionated nerves (as the root in question will be seen below to be), must be considered now to be merely a physiological and not an anatomical one. It is obvious, from more recent investigations, that the only cells with which the fibres of such roots are directly connected are those of the peripheral ganglia belonging to these roots.

This being the case, it will be convenient to denominate such internal nuclei, whose cells are not directly continuous with the root fibres, "terminal nuclei," in distinction to those nuclei whose cells are in direct continuity with root fibres (ganglia of sensory roots and motor nuclei), which may be called "nuclei of origin." This distinction and terminology is that adopted by Kölliker in his *Gewebelehre* (34) ("Endkern" and "Ursprungskern").

In the tadpole it is difficult to determine whether some of the acoustic fibres may not be similar to those of the dorsal VII. From appearances in the tadpole, and from the fact that in the frog the origin of a portion of the VIII is ascribed to similar ganglion cells (Köppen), even from the mere fact of the persistence of these cells after the disappearance of the dorsal VII, this similarity might be inferred.

This root, the dorsal VII, already reduced in the tadpole, disappears in adult Anura. The reasons for this disappearance will become evident when its distribution is considered. It may be well to point out here that this fact has caused some confusion and apparent discrepancy in the accounts of the Facialis by different observers, some of whom assert the origin of the VII to be dorsal to the VIII, while others assert it to be ventral. The latter refer to forms in which the dorsal VII has disappeared; the former overlook the ventral portion of the VII. Köppen (35), however, overlooking the ventral portion in the frog, in which the dorsal root is lost, is obliged to look for the VII in the Trigemini. He is further misled by expecting to find the Facialis a purely motor nerve.

At $852 \pm$ the ventral roots of the VII separate from the VIII, the coarse-fibred root (VII *ab*) forming the outer and lower portion of the bundle. The description of the dorsal VII will be taken up first.

The Dorsal VII ("VII b").—When this separates from the dorsal side of the VIII, a portion of the latter intervenes between it and the ventral VII (VII *aa* + VII *ab*). As the VIII passes into the auditory capsule the V emerges from the medulla, so that there is only a narrow space between the V and the VIII. Into the upper part of this space the dorsal VII is wedged; into the lower part the ventral VII (VII *aa* + VII *ab*) (Pl. X, Fig. 25). The fibres of the ventral half of the dorsal VII soon begin to bend ventrad, and at 842 separate from the dorsal half. Caudad of this a few fibres pass ventrocephalad from this ventral half to unite with some fibres detached from the ventral VII. The ventral half of the dorsal VII, after its separation from the dorsal half, passes ventrad between the V and VIII nerves, and comes to lie ($832 \pm$) immediately above the ventral VII, with which it unites. It here lies immediately above the bundle of coarse fibres, VII *ab*, occupying the outer side of the ventral VII (Pl. X, Fig. 25). The union of these two bundles now becomes so close that they can only with some difficulty be distinguished; but the course of their respective fibres can be stated with considerable certainty from three other grounds also, *viz.*, the distribution of the dorsal half of the dorsal VII; the nature of the fibres of certain branches in the distribution of the ventral VII + $\frac{1}{2}$ dorsal VII; and the relations of homologous bundles in Amblystoma. Owing to the close relations of this portion of the dorsal VII to the ventral VII its further course can, however, be most conveniently described in connection with the latter.

The *dorsal half of the dorsal VII* remains close to the V, and as it proceeds cephalad comes gradually to lie immediately above it. At 775 it becomes ganglionated, and at 751 divides into two nearly equal parts.

The lower of these divisions ($\frac{1}{2}$ VII *b*₂) passes ectad just beneath the anterior extremity of the auditory capsule and above the Gasserian ganglion. About here it gives off a twig of a few fibres ($\frac{1}{2}$ VII *b*_{2a}, 732), which can be traced ectad to lateral line sense organs lying in the skin some .15 mm. caudad of the caudal surface of the eyeball.

The main nerve, proceeding further ectad, soon gives off another twig of several fibres ($\frac{1}{2}$ VII b_{2b} , 727). This twig immediately fuses with a fine-fibred twig, also very minute, which is given off by the largest and outermost of the accessory trigeminal branches described above (p. 17) as issuing from the anterior extremity of the Gasserian ganglion. These two bundles of fibres thus brought together are distinguishable from each other, owing to their difference in fibre calibre, and soon separate, the trigeminal portion going caudad and dorsad, and coming in contact temporarily with a division of the middle of the accessory trigeminal twigs just above the outer edge of the auditory capsule. The facial portion proceeds ectad and has a distribution near and probably similar to the preceding facial twig, though it could not be so clearly traced.

The main nerve ($\frac{1}{2}$ VII b_2) continuing ectad comes in contact (726) with the outer of the accessory trigeminal branches. This contact and temporary fusion is a peculiar one: the facial passes through the trigeminal branch, each, however, seeming to preserve its continuity. At this point of contact each gives off a twig. That from the trigeminal portion is fine-fibred, and fuses—temporarily, apparently—with the facial twig ($\frac{1}{2}$ VII b_{2c}). The fibres of these very minute trigeminal twigs are so fine that it is not possible to ascertain in ordinary preparations whether some fibres may not remain with the facial twigs. The main trigeminal branch now breaks up, and supplies the skin of the region just ectad of the posterior surface of the eyeball. The facial twig ($\frac{1}{2}$ VII b_{2c}) divides. One part crosses and temporarily fuses with a trigeminal twig (700-687), proceeding cephalad parallel with the principal facial branch. Some of its fibres are traceable to a large lateral line sense-organ in the epidermis, just below the cornea (625). The remaining four fibres proceed further cephalad and innervate a lateral line sense-organ cephalad of the latter and in a similar position (588). The other part of this facial twig could not be completely traced to lateral line sense-organs.

The main branch ($\frac{1}{2}$ VII b_2), after giving off these twigs, bends forwards, proceeding cephalad under the eye. At intervals it gives off small twigs of only a few fibres each. Many of these

could not be traced into the lateral line sense-organs. When this was the case, the cause must be sought in defects in the preparations, especially where the twigs are so excessively minute. It may be reasonably inferred that all of these twigs end in these organs, whose line the main facial branch follows so closely, especially as an organ is always found in the vicinity of a twig though the complete connection be not present. This branch can be followed, gradually diminishing owing to the separation of twigs, nearly to the very anterior extremity of the head.¹

Returning to the other subdivision of the dorsal VII ($\frac{1}{2}$ VII b_1 , 751), this proceeds at first directly cephalad just mesad of the lower part of the anterior extremity of the auditory capsule, gradually leaving the Gasserian ganglion. As it separates from the latter it receives a small, fine-fibred twig, which can be traced around the Gasserian ganglion to the sympathetic. Continuing cephalad and dorsad it is joined on its ventral side (694) by the innermost of the three accessory trigeminal branches. It here (687) gives off a twig from its dorsal side which proceeds caudad and ectad ($\frac{1}{2}$ VII b_{1a}) coming in contact (717) with a division of the middle of the three trigeminal branches, which proceeds to meet it. The subdivisions of the latter pursue courses in part parallel to twigs of the facial, *i.e.*, both proceed ectad and caudad, the facial supplying one (or more) lateral sense-organs lying above the space between the anterior extremity of the auditory capsule and the posterior surface of the eyeball, and also above the latter. Thus a part of the middle of these trigeminal branches is connected with one subdivision of the dorsal VII, and another part with the other subdivision (Pl. XII, *A z*).

The innermost of these three trigeminal branches, after coming in contact with the subdivision of the dorsal VII ($\frac{1}{2}$ VII b_1 , 694), passes dorsad along the inner side of the latter, fusing temporarily with it. From the ventral side of the facial branch a portion of the latter separates. This branch

¹ These sense organs in the tadpole, as is well known, are noticeable on the exterior as rows of light dots. This appearance is owing to the absence of pigment among their cells. They are not enclosed in canals, and each one is usually slightly depressed.

($\frac{1}{2}$ VII b_{1b}) proceeds cephalad parallel to the main facial branch and finally reunites with it (560), without having, apparently, in the meanwhile, given off any fibres.

The main subdivision of the dorsal VII ($\frac{1}{2}$ VII b_1) continues cephalad along the dorsal surface of the head and inside the eye to the extremity of the head. At intervals it gives off twigs similar to those of the other subdivision; like them, evidently supplying the line of lateral sense-organs in this region.

The parallelisms in courses between the trigeminal and facial twigs above described are very striking, and are often observed even in very minute ramifications. The significance of these parallelisms will be discussed below.

Somewhat more light is thrown upon the relations of these trigeminal and facial branches by means of Golgi preparations. The two facial branches, as they separate from the Gasserian ganglion, seem to be composed exclusively of the coarse, heavily medullated fibres so characteristic of them, nor do both, or even one of them, always appear to receive directly a sympathetic twig.

The trigeminal branches, however, contain, besides the medullated fibres of varying sizes as already noted, a number of fibres which do not appear to be medullated and are impregnated. These appear to have rather the character of *nervi nervorum* and some of them, at least, though apparently not all, can be traced to the *sympathetic*. When fusions take place with the facial branches, though the integrity of the two branches is in the main preserved, yet a number of these fine fibres pass from the trigeminal to the facial twig and join the latter.

The ventral VII + $\frac{1}{2}$ the dorsal VII (VII aa + VII ab + $\frac{1}{2}$ VII b) consists, as above stated, of three components: one (VII ab), the most ventral in derivation, from a motor nucleus (or the posterior longitudinal fasciculus or both), one (VII aa) from the fasciculus communis and the third ($\frac{1}{2}$ VII b) from the ventral half of the dorsal VII which later joins the two former. Where the first two components are fused with the VIII the ventral root (VII ab) comes to occupy the outer position and

forms a prominence on the ventral side of the Acusticus just mesad of the foramen for the entrance of the posterior branch of the latter into the auditory capsule. The ventral VII finally separates from the Acusticus (852), occupying the position previously described (p. 116), and is joined by the ventral half of the dorsal VII. This and what will be called hereafter the motor root (VII *ab*) occupy the outer side of the nerve, the former ($\frac{1}{2}$ VII *b*) lying above the latter (VII *ab*). The whole nerve lies close to the ventral side of the Trigemini in contact with it but not completely fused with it, *i.e.*, there is always visible a line of demarcation between the two.

At $802 \pm$ the inner, fasciculus communis bundle (VII *aa*) begins to slip ventrad past the other two so that the greater part of it comes to lie below them instead of on their inner side. It then soon becomes ganglionated (788). This ganglion occupies the extreme ventral part of the V + VII, lying below the other two facial components ($\frac{1}{2}$ VII *b* + VII *ab*). It attains its greatest dimensions when the Gasserian ganglion proper is just beginning to appear in the transverse sections, *i.e.*, one half of it lies caudad as well as ventrad of the Gasserian ganglion proper. Its anterior part is fused with the ventral side of the Gasserian ganglion, cephalad of 767, so that it is somewhat difficult to distinguish between them at this point. Finally the fibres of the fasciculus communis bundle are seen emerging ($760 \pm$) from the ventral part of the ganglion and form the *R. palatinus facialis*. In about the same transverse plane the other two components ($\frac{1}{2}$ VII *b* + VII *ab*) begin to pass ectad here from the Gasserian ganglion, a portion of them, presumably that from the dorsal VII, which may now be called the lateral line component, having first come into connection with ganglion cells. They evidently separate, occupying the same relative positions, *i.e.*, the lateral line component uppermost. As they separate from the ganglion these two components receive on their ventral side a bundle from the ganglion of the fasciculus communis component (VII *aa*, Pl. X, Fig. 26). The branch of the Facialis thus constituted is the *R. hyomandibularis*. The *R. palatinus VII* is thus composed of the bulk of the fasciculus communis component (with possibly the addition

of some trigeminal fibres and also of fibres from the sympathetic), while the *R. hyomandibularis* comprises the ventral half of the dorsal VII, the motor root and a part of the fasciculus communis component ($\frac{1}{2}$ VII *b* + VII *ab* + part of VII *aa*). The possibility of its having also received some fibres, not many, from the Trigemini cannot be excluded, however, and it also receives fibres from the sympathetic.

The course of the *R. palatinus VII* along the roof of the pharynx is sufficiently indicated in the chart. There are some features in its branching, however, which deserve special attention.

At $400\pm$ it gives off a branch which proceeds directly mesad and innervates a curious fold which extends transversely across the roof of the pharynx. This fold, which is described also by F. E. Schulze (53), is directed cephalad and is partially continuous laterally with the surrounding pharyngeal epithelium so as to form a shallow pocket opening anteriorly. This is the way it appeared also in another tadpole examined macroscopically. In the two figures of this fold given (Pl. VIII, Figs. 8 and 10) one is taken from a section through its posterior part so as to pass tangentially through its epithelium. The other is from the next section cephalad. In other sections examined this fold appears to be more free and to be directed posteriorly. On and around the fold are a number of end buds (taste bulbs), as are described below, which are innervated by the branch of the *R. palatinus* just mentioned. This fold, as the figures seem to demonstrate, is especially richly innervated. Nor is this due merely to an inequality in the impregnation as is shown by the presence of the branch from the main trunk, the largest branch given off by the *R. palatinus* up to this point.

The location of this branch corresponds with that of the vomerine teeth in the adult, possibly being slightly caudad of the latter.

According to Wiedersheim, in the frog the epithelium in the vicinity of these teeth is supplied with taste bulbs (quoted in 17).

At the same place where the branch just described is given off, another larger branch separates from the *R. palatinus*,

proceeds cephalad and, turning ectad, becomes continuous with a branch of the R. maxillaris V as already described (p. 111). The remainder, much diminished, passes on cephalad and becomes continuous with a branch of the R. ophthalmicus V as already described (p. 108).

From the nature of these curious anastomoses, it is difficult to determine exactly where the Facialis ends and the Trigemini begins. Their significance lies, I believe, in the fact that the R. palatinus VII, on the one hand, and the R. ophthalmicus and maxillaris V, on the other, innervate territories morphologically distinct, and that in the region where these fusions occur these two territories meet. In other words, they occur just about on the boundary between the pharynx and the stomodaeum. Of the part becoming continuous with the R. ophthalmicus, it is not improbable that all which continues cephalad beyond this commissure, though indicated as partly composed of each element in the chart (*q.v.*), belongs to the Trigemini, and that the same is true of a considerable portion of that which is caudad of this point of fusion. Along its course this branch gives off a considerable number of fibres which form a plexus around the openings of the posterior nares into the pharynx. As a line drawn through the anterior part of these openings would indicate the line of demarcation between the stomodaeum and pharynx, it is not impossible that these fibres represent the last fibres from the R. palatinus proper, and that the remainder of the branch consists entirely of fibres coming caudad from the R. ophthalmicus. A still more minute study of this portion, however, would be necessary to determine this point exactly, from observation, and the possibility must also be admitted that the regions innervated by fibres from the R. ophthalmicus V and the R. palatinus VII, respectively, overlap to some extent. This, however, would not impair the general validity of the view here put forward as to the significance of these anastomoses.

What has been said above applies also to the anastomosis between the R. palatinus VII and the R. maxillaris V. Here again the exact delimitation of the R. palatinus cannot be determined.

It may be remarked here that it would be extremely interesting to study, from this point of view, the innervation of the stomodaeum and pharynx of forms in which their relative extents vary. Indeed, this will probably be necessary as supplying one of the guides in reaching accurate knowledge of the homologies of the nerves of this region in different forms, and especially with the higher forms, where the relations are so complicated.

The mode of branching of the R. palatinus VII in the adult frog, and also its terminations in epithelium, glands, and blood vessels, have already been described by Stirling and Macdonald (60). These investigators made use of the gold method, and it will be well to add some results obtained with the Golgi method, especially as the endings in this region have not been so fully described in the tadpole by Retzius (51), von Lenhossék (38), and others, as in other forms.

The structures innervated are blood vessels, general epithelium, glands, and end buds (taste bulbs). In the olfactory region we have the mucous glands, which fall, in part at least, in the trigeminal territory. My impregnations, however, have not demonstrated much respecting the innervation of these glands, merely showing some scattered fibres coursing around their periphery.

Vaso-motor fibres, following the blood vessels and often ending in their walls with little knob-like expansions, are met with here and there. Whether these vaso-motor fibres come merely from sympathetic fibres mixed with those of the R. palatinus proper, or also from the latter, it is hardly possible to determine. Stirling and Macdonald have described nerve cells in this region with spiral fibres. I have also occasionally met with nerve cells, though whether of this type or not I could hardly determine.

Before treating further of the finer terminations of this nerve, and in order to make clearer some points mentioned below respecting the terminations of other nerves, it may be well to indicate briefly the structures found in this region. These have been described so clearly and admirably by F. E. Schulze (53) that I cannot do better than give a brief résumé of a

portion of his description. Schulze divides the roof of the stomodaeo-pharyngeal cavity into five regions. These regions are demarcated by certain folds and elevations or papillae. The anterior region or field is that portion lying in front of the transverse fold already mentioned; the middle field, free from any high papillae, is bounded in front by the transverse fold, laterally by a row of high papillae, and posteriorly by a fold. On each side of it are the lateral fields characterized by the presence of high papillae. The fold which forms the posterior boundary of the middle and lateral fields has a scalloped outline and extends transversely across the cavity. Behind it is the posterior field, in which the character of the epithelium changes, being destitute of papillae and studded anteriorly with "multicellular glands," as they are designated by Schulze. Posteriorly this epithelium merges into the oesophageal epithelium. The papillae are elevations of the epithelium, the interior being composed of connective tissue, and they bear one or more "taste bulbs." The latter are found as well between the papillae, and also, according to my observations at least, in the posterior field. The multicellular glands are composed of a number of appressed elongated cells forming a cup-shaped structure whose concavity forms a shallow depression in the epithelial surface, and whose convexity projects slightly into the subepithelial connective tissue.

The floor of the stomodaeo-pharyngeal cavity is divided, according to Schulze, into five similar regions, the rudiment of the tongue marking the boundary between the anterior and middle fields. Here also is a similar transverse scalloped fold marking off the posterior field. In the posterior field the gill cavity opens and is partly covered by the folds formed by the posterior field, *i.e.*, the anterior and posterior velar folds ("Kiemendeckplatten"). On these folds the above mentioned glands are so numerous that they form a continuous layer without intervening indifferent epithelium, a condition which is approached also on the posterior field of the roof in places. It is on the edges and under side of the velar folds that these glands are so numerous, and I may add, from my own observations, that those on the roof of the pharynx are so grouped as

to be most numerous always over the opening into the gill cavity. In those parts of the roof not directly above this opening they immediately dwindle away.

The nerve fibres, in my preparations, form beneath the epithelium a dense plexus from which fibres pass upward into the epithelium. In the thinner, indifferent epithelium, which seems usually to be the least richly innervated, they run among the cells irregularly, but do not as a rule seem to penetrate more than about two-thirds through its thickness towards the surface.

A number of nerve fibres approach the base of the *taste bulbs* and there break up, forming a dense structure (Pl. IX, Figs. 15, 16, and 24), often, apparently, more of a granular than fibrous character, and at times staining less black than the nerve fibres. This structure evidently corresponds with that described by von Lenhossék in fishes (38) and termed by him the cupula. From this structure nerve fibres arise which ramify around the bud, often rising nearly to its peripheral surface. Whether they also penetrate between the cells of the bud it is rather difficult to determine.

The nerve fibres passing immediately below the *multicellular glands* send at right angles vertical fibres up into them or close around them. The course of these fibres varies somewhat. In some preparations they rise nearly parallel with each other almost to the very surface of the epithelium where they end in little knobs either among the cells of the bud or immediately around them (Pl. IX, Fig. 17). In other cases the fibres rise more irregularly, and when they have penetrated into the upper third of the epithelium they turn and branch so as to form a dense ring-like plexus apparently encircling or penetrating the gland at this level. From this plexus a number of nearly parallel fibres pass upwards converging towards the central axis of the gland and end in enlargements in or very near the surface of the epithelium (Pl. IX, Figs. 18 and 19). I am inclined to believe that the latter fibres, at any rate, penetrate into the gland near its free surface. Transitional forms are abundant where the fibres from the subepithelial plexus pass upward more as in the first mode of termination, but branch

near the surface to a certain extent before terminating (Figs. 20 and 21). These terminations are often not simply rounded knobs but have a more elongated club shape and are somewhat irregular in outline (Figs. 18-21). As a number of fibres press in from all sides towards the centre of the depression in the epithelium formed by the gland, this locality is quite filled with these bodies. The irregularity and size of these enlarged terminations may be due to some irregularity in the staining, but since they occur often in the cleanest impregnations and since expansions of even greater size exist elsewhere, *e.g.*, in terminations in muscles, they may be considered true pictures.

These appearances in the glands seem to me to be not easily reconcilable with Dogiel's (14) denial of free endings and assertion of the prevalence of a closed network as the terminal apparatus. It is, of course, possible that these enlargements which lie immediately below the surface, almost in it, are not the final terminations and that there are always, *e.g.*, unstained transverse fibres connecting them and forming closed meshes. It is also true that the various methods of staining nerve fibres, especially the Golgi method, are irregular and incomplete in their action, yet it is not likely that the latter would always omit certain fibres such as these hypothetical ones. The manner in which these fibres terminate negatives still more strongly their existence. That true anastomoses *may* occur is not to be denied and sometimes the appearances favor their existence (*vide supra*) but they can hardly be of universal occurrence. Neither physiologically nor embryologically would there seem to be any special reason for their existence in such peripheral structures as epithelium, though they might easily occur now and then owing to secondary fusions.

The *R. hyomandibularis facialis*, as described above, leaves the Gasserian ganglion at about the same transverse plane as the *R. palatinus* ($760 \pm$). As it leaves, it is composed, as has been seen above, of three components, occupying the nerve in the following order: The most dorsal is the dorsal VII component ($\frac{1}{2}$ VII *b*); next to this is the motor component (VII *ab*); and most ventral is the fasciculus communis component (VII *aa*) (Pl. X, Fig. 26). The destination of the first and last compo-

nents can be traced with certainty ; that of the second one is not so easily made out, as will be seen below. This is partly owing to the fact that the fibres of VII *b* are remarkable for their uniform coarseness and heavy medullary sheath, and those of VII *aa* for their fineness and very thin sheath. On the other hand, those of VII *ab*, though tolerably coarse and well sheathed, do not possess such well-marked characteristics.

As the R. hyomandibularis proceeds ectad, flattened out under the quadrate, VII *aa* comes to occupy the inner side of the nerve, next to VII *ab*, while outermost is VII *b*, which also overlies VII *ab*. This is about their relation when joined by the communicating branch from the IX + X (690), and is shown in Fig. 27. This latter branch occupies, as is seen in the figure, the outer third of the joint nerve. It is composed of mixed fibres, similar to those of the Trigemini.

As the R. hyomandibularis emerges from beneath the cartilage it acquires a round outline, as seen in transverse section, but the relations of the components are much the same, except that the portion from the IX + X takes up a somewhat more dorsal position, pushing mesad $\frac{1}{2}$ VII *b*. In Fig. 29 a small twig is seen leaving the ventral side of the nerve and deriving its fibres from VII *ab* (483). This twig passes ectad close under the nerve, and after proceeding cephalad a short distance enters a vertical muscle just outside the nerve, which is identified as the M. orbitohyoideus described by F. E. Schulze (54). A twisting and flattening of the nerve now takes place, so that the positions seen in Fig. 30 are assumed. VII *aa* is now uppermost, $\frac{1}{2}$ VII *b* and VII *ab* next, while the component from the IX + X is ventral. Along in this part of the nerve it is at times difficult, for the reasons mentioned above, to trace VII *ab*. There is little doubt, however, but that its course is as given here.

As indicated in Fig. 30, the nerve divides into two parts. The ventral of these two divisions, the R. *hyoideus*, is composed largely of fibres from the IX + X, with the addition of a bundle from VII *ab*. It proceeds ventrad (433) and caudad through the M. orbitohyoideus, and then turns mesad. It then divides (508), as indicated in the chart, one part supplying the

M. mylohyoideus posterior (= submaxillaris post. = intermaxillaris post. = subhyoideus) and the other supplying the skin below this muscle. There is, thus, quite a marked resemblance to the branch of the R. mandibularis V which innervates the M. mylohyoideus anterior and the skin below it. The R. hyoideus, however, is composed of fibres from *two* nerves, the VII and the IX.

While it is difficult to distinguish the two sets of fibres in the R. hyoideus VII, yet it is probable, from direct observation alone, that the fibres from VII *ab* are those which innervate the muscle.

It is obvious from the preceding that the cutaneous part of the R. hyoideus does not belong to the lateral line system of nerves to the head, and belonging to the VII. As this cutaneous component is derived from the IX + X, it will be further treated below.

The remainder of the R. hyomandibularis proceeds cephalad, and soon divides again (368). Of these two divisions the ventral one comprises the remainder of the communicating branch from the IX + X and $\frac{1}{2}$ VII *b*, while the dorsal one comprises the remainder of VII *ab* and all of VII *aa* (fasciculus communis). The former is the *R. mandibularis externus*. It proceeds ventrad just inside the M. orbitohyoideus. The bulk of the coarse fibres belonging to $\frac{1}{2}$ VII *b* have become aggregated on its outer side. As the nerve emerges on the ventral side of the muscle it becomes divided into two branches, an inner and lower (mand. ex. *a*), composed of the finer fibres of the IX + X component, together with a few coarse fibres, and an outer branch (mand. ex. *b*) comprising the coarse fibres of $\frac{1}{2}$ VII *b*, together with a portion also of the finer fibres of the communicating branch from the IX + X.

The former of these two branches (mand. ex. *a*) sends off a small twig, which is reinforced by fibres from the other branch, and supplies the skin. The remainder, in every way resembling the cutaneous portion of the R. hyoideus, proceeds caudad and ventrad, and is distributed to the skin of the ventro-lateral aspect of the body in this region. No connection was observed between it and lateral sense organs. It is a "general cutaneous"

branch. This branch is probably the representative of the R. auricularis VII in the frog (17).

The latter of these two branches, *i.e.*, the outer (mand. ex. *b*) soon divides. The upper (dorsal) of these two subdivisions (mand. ex. *b*₁) gives off twigs, containing the smaller fibres, to the skin. The bulk of the remainder, consisting of coarse fibres, proceeds caudad, supplying a line of lateral sense organs along the side of the head ventrad to those under the eye. The larger ventral subdivision (mand. ex. *b*₂) proceeds caudad, parallel with the other, apparently supplying no lateral sense organs until the dorsal subdivision has terminated. It also appears to gradually lose its finer fibres. It divides at 585. One part, proceeding caudad and dorsad, supplies lateral sense organs with its coarse fibres. The other part proceeds caudad and ventrad to about 800, where it bends mesad, proceeds transversely across the ventral aspect of the body, and probably supplies a line of lateral sense organs present in this region. There is, in addition to these branches of the R. mandibularis externus, a small branch (mand. ex. *c*) which proceeds cephalad, turning ventrad and caudad, as indicated in the chart.

The dorsal of the three principal divisions of the R. hyomandibularis divides. The ventral of these two divisions contains nearly all the remaining fibres of VII *ab*, and soon enters (357-346) the two muscles lying just mesad of the nerve. A few of the coarse fibres of VII *ab* remaining with the dorsal (fasciculus communis) division separate and also enter these muscles. Of these two muscles, one is identified as representing the Mm. suspensorio-angularis and quadrato-angularis described by Schulze (54). It appears to be one muscle here, though there are some evidences of a separation into two. The other muscle is the cerato-hyo-angularis of Schulze. The behavior of these two sharply contrasted bundles of fibres (VII *aa* and VII *ab*) and the manner in which the coarse ones are, as it were, picked out to innervate the muscles, is very interesting and instructive. The remainder of the dorsal division of the R. hyomandibularis consists now of a bundle of fine, lightly-staining fibres, among which are a number of deeply-stained but small fibres. By tracing it out we have seen that

this bundle represents the fasciculus communis component. When it separates from the R. hyomandibularis it is the *R. mandibularis internus facialis*. This nerve now proceeds cephalad and bends mesad under the quadrate ($310 \pm$) to the angle of the pharynx. It proceeds cephalad along the pharynx, supplying some fibres to its mucous membrane. Its main distribution is not general, however, but is principally to one locality. This is shown in Pl. VIII, Fig. 11. A great number of the fibres are here seen to supply a large papilla or elevation of the epithelium about at the boundary between the pharynx and mouth. A number of fibres proceed mesad to innervate the floor of the pharyngo-oral cavity. This is in the same transverse plane as the location of the future tongue, as is shown by the N. hypoglossus and R. lingualis glossopharyngei. I have examined sections of the frog also, with reference to this point, and find that the termination of this nerve, the R. mandibularis internus facialis, is in the region of the anterior part of the attachment of the tongue, and that a part of it, at least, seems to send branches into the anterior part of the tongue itself.

It may be well to point out here that these observations confirm, in most respects, what would be considered *a priori* as the most probable destinations of these components. As the dorsal division of the dorsal VII (VII *b*) was found to supply sense organs of the lateral line system, a similar destination would be the natural supposition respecting the distribution of that ventral portion going over into the R. hyomandibularis. Again, as one portion of the fasciculus communis component (VII *aa*) was found to be distributed, as the R. palatinus VII, to the roof of the pharynx, it is most consistent that the portion entering the R. hyomandibularis should likewise supply a portion of the pharynx. By elimination merely, this would leave the third component, *i.e.*, the *motor root* (VII *ab*), and also the R. communicans IX ad VII to supply the general cutaneous (*i.e.*, cutaneous excluding the specialized lateral line system) and motor branches. VII *ab* has the position and characteristics of a motor root, and is the one which would be considered most likely to enter the motor branches. In this

connection an observation of Volkmann (64) may be cited. Volkmann found, by stimulation, that the communicating branch from the IX + X did not contain motor fibres.

These observations are partially checked in another way by a most beautiful extirpation experiment performed by nature. In the frog the lateral line system of sense organs disappears and, as I have verified myself in the common frog, in the toad, and in one of the Hylidae, the dorsal VII (VII *b*) disappears also. Consequently the various rami (see table below) derived from this root are wanting in these forms, but on the other hand, such cutaneous branches as do not belong to this system persist. Accordingly we find in the frog, for example, a cutaneous branch from the R. hyoideus. Furthermore, this affords a firm basis in determining the R. auricularis VII (17) and in excluding, as possible homologues, those cutaneous branches derived from the dorsal VII.

The question of certain homologies is discussed in another portion of this paper. Some, however, are assumed in the table given here, which presents in a convenient form an analytical summary of the N. facialis.

I may add that an examination of serial sections through the brain and proximal portions of the nerves of the frog, stained by Weigert's method, show very clearly the two components of the VII, before joining the V, namely, on the outer side the coarse-fibred motor part and mesad the fine-fibred fasciculus communis component. Although somewhat out of place here, it may be remarked that a bundle of fibres can be traced in the V which preserve their integrity through the Gasserian ganglion entering the R. mandibularis, and which, tracing them proximad, appear to be derived from the motor root of the V.

In *Amblystoma* larvae the relations of these roots are somewhat different. They are shown in the figure occupying the corner of the chart (Pl. XII, C). Here the principal difference lies in the fact that the VII is not pushed forward into the V. There are also other differences: in the *Amblystoma* larva the dorsal VII is considerably larger than in the tadpole, while the Auditory is smaller relatively, not only to the dorsal VII but to the medulla. Furthermore, it is separated from

the dorsal VII by an interval; and in that interval, and dorsal to the Auditory, emerges the fasciculus communis root.

"In *Amblystoma* (Pl. XII, C) the fasciculus communis component of the VII, shortly after emerging from the medulla, enters a ganglion lying partly under the anterior extremity of the auditory ganglion and partly anterior to it. From the anterior end of this ganglion emerges the R. palatinus, which then dips downwards piercing the roof of the mouth and proceeding forwards along the latter. Laterally to this and also from the ganglion near its extremity, a small branch is given off which passes directly outwards and forms the most ventral portion of the trunk of the VII. It soon separates, proceeding outwards and downwards to the side of the oral cavity, where it proceeds forwards along it and along the inner side of the lower jaw. It is thus in every respect, except that it is not pushed forwards into the V, identical with the R. mandibularis of *Anura*, thereby confirming the view that the latter is derived from the fasciculus communis.

"The ventral root, or rootlets, of the VII (VII *ab*) could, in part at least, be traced out, and forms the portion of the trunk of the VII, as it passes outwards, which lies immediately above the fasciculus communis branch just described. Though fused with the latter and with a part of the dorsal VII (VII *b*) mentioned below, it can yet be traced continuously until it likewise separates and is distributed to the Mm. digastricus and mylohyoideus posterior, corresponding to the motor branches of the R. hyomandibularis in *Anura*, and showing the derivation inferred for them in the latter to be correct.

"The dorsal VII (VII *b*) divides soon after its exit, part passing directly cephalad along the dorsal side of the Gasserian ganglion. It is here this part enters its ganglion, which is fused with the Gasserian ganglion proper. The ventral half of the dorsal VII passes downwards as well as forwards, and then bends outwards directly under the auditory capsule and just anterior to the entrance of the anterior branch of the VII into the capsule. It here fuses with the other components of the VII mentioned, forming the dorsal part of the trunk. In this part of its course and under the outer edge of the auditory

ANALYTICAL SUMMARY OF THE FACIALS.

| Roots (components). | Branches. | Distribution. |
|---|---|---|
| Dorsal VII (VII <i>b</i>) { 1. Dorsal half (ganglionated) 2. Ventral half (ganglionated) | { R. ophthalmicus superficialis VII. R. buccalis, R. oticus and other small branches part of R. hyomandibularis (<i>q. v.</i>). } | Supra-orbital line of sense organs. Infra-orbital line of sense organs. |
| Fasciculus communis (ganglionated, VII <i>aa</i>) | { R. palatinus part of R. hyomandibularis (<i>q. v.</i>). } | Roof of pharynx. |
| Motor root (VII <i>ab</i>) | part of R. hyomandibularis (<i>q. v.</i>). | |
| Components. | Branches. | Distribution. |
| ½ dorsal VII Fasciculus communis Motor root | R. mandibularis externus R. mandibularis internus Rr. not named part of R. hyoideus part of R. hyoideus Communicating branch from IX + X R. auricularis + | Lateral sense organs, mandibular and opercular. Floor of anterior pharynx. Mm. orbitohyoideus, suspensorio-angularis, quadrato-ang. and cerato-hyo-angularis. M. mylohyoideus posterior. Skin ventrad of M. mylohyoideus posterior. Skin in vicinity of ear. |
| R. hyomandibularis | | |

capsule it passes into its ganglion, composed of large ganglion cells. It is a cutaneous nerve; and probably, as in the tadpole, supplies the lateral sense-organs. These are here more irregularly arranged.

"Thus the dorsal VII has two ganglia, one on each of its two main divisions, while the fasciculus communis component has one at its point of forking. The motor portion (VII *ab*) is, of course, non-ganglionated."—*Quoted from* (62).

The Abducens.—As this nerve enters into close relations with the VII and V, that part of its course connected with them will be described here. It makes its exit from the ventral side of the medulla in the same transverse plane as the exit of the second root of the IX + X. It proceeds ectad and curves cephalad lying a short distance mesad of the sympathetic nerve. At 912 it crosses the sympathetic, now lying ectad of the latter. At 806 it comes in contact with the ventral side of the VII. It is imbedded in the mesal side of the ganglion of the fasciculus communis component of the VII. Emerging from the anterior end of this it separates from the R. palatinus VII as the latter passes ventrad through the roof of the mouth, lying immediately above the R. palatinus (747±) and below the ganglion of the R. ophthalmicus trigemini. It now unites with the ventral side of the R. ophthalmicus trigemini (734), slipping around to the outer side of the latter. There seem to be ganglion cells in connection with it (703), although these may belong to the oculomotor nerve. The VI leaves the R. ophthalmicus (697), and divides, the larger part proceeding cephalad and ectad to the M. rectus externus, which it innervates.

It may be as well to restate here concisely the relation to the Gasserian ganglion proper of the various nerves and ganglia which are connected with it.

Running along the dorsal side of the Gasserian ganglion proper is the dorsal half of the dorsal VII. Its ganglion is fused with the dorsal side of the Gasserian ganglion. Next, ventrally, are the ganglia of the Rr. maxillo-mandibularis and ophthalmicus trigemini, which compose the great bulk of this ganglionic mass, and constitute the Gasserian ganglion proper. The ophthalmic portion—mostly indistinguishable from the

other — seems to occupy more the anterior portion of the ventral side. Occupying the posterior portion of the ventral side we have the ganglion of the fasciculus communis component of the VII, from which come the R. palatinus VII and the smaller branch which goes over into the R. hyomandibularis VII. In the outer side of the whole ganglion, and between the Gasserian ganglion proper and the ganglion of the fasciculus communis component, there are ganglion cells which must be regarded as constituting the ganglion of the ventral half of the dorsal VII, which subsequently forms part of the R. hyomandibularis. Besides these ganglia, we have also non-ganglionated bundles of fibres. There is the bundle of motor fibres of the portio minor of the Trigemini, passing through the Gasserian ganglion. There is also the motor bundle of the VII which passes, with the ventral half of the dorsal VII, through the outer side of the ganglion. The course of the Abducens has been described. It is in the lower part of the inner side of the whole ganglion. Finally, there are sympathetic twigs which join the various branches emerging from this ganglionic complex (see pp. 118, 119, and 151).

3. *The Glossopharyngeus and Vagus.*

The determination of the composition of these nerves presents considerable difficulty in the tadpole, principally because of the manner in which they are, as it were, pushed together and out of position by the auditory capsule. Following the different roots through the ganglia accurately can hardly be accomplished, yet I believe the general nature of these ganglia is as described below. Some of the peripheral branches also have not been traced as completely as could be desired.

Five roots can usually be distinguished composing the IX + X. They are represented on the chart somewhat spread out, in some particulars, for clearness. The *first root, i.e.*, that one most cephalad, emerges from the medulla at 911. It leaves the medulla at a more dorsal level than the other roots and, as seen in the chart, as it approaches the medulla it

curves cephalad. The fibres of this root are exclusively coarse, very uniform in size, and apparently have an internal origin similar to those of the dorsal VII, which they resemble in every way. At 926 it separates from the medulla and proceeds ecto-caudo-ventrad a short distance when it is joined on its ventral side by the second root of the IX + X.

The *second root* emerges from the medulla (936) at some interval from the first and, as mentioned above, at a lower level (Pl. XI, Fig. 37). The bulk of its fibres are derived from the fasciculus communis. It is joined, however, on its ventral side by a slender bundle of fibres which, as they penetrate further into the medulla, curve ventrad and have an origin considerably further inward and ventral to that of the rest of the root. They, apparently, are connected with a group of cells, but no actual continuity with processes of cells was observed. These fibres are coarser than those from the fasciculus communis. This is, undoubtedly, a motor rootlet and seems to be comparable in position and general characteristics with the motor roots of the Trigemini and Facialis. It is not represented in Fig. 37, which is drawn from a Golgi preparation in which the fibres of this ventral rootlet were not impregnated. As will be observed in the figure, the fibres of this second root break through the ascending Trigemini tract in order to reach the exterior.

The *third root*, preceded sometimes by a minute intermediate rootlet, often emerges in close juxtaposition with the second root. This root has, at least, a threefold origin. As shown in Fig. 36, the dorsal position of this root is derived from the ascending tract of the Trigemini. This derivation is, undoubtedly, contrary to the views generally held as to the origin of the IX + X, but is, I believe, shown to be correct not only by a study of the origin of the roots themselves but also by the nature of certain of the branches of the IX + X as described hereafter. As seen in Fig. 36, these fibres come from the lower part of the ascending Trigemini tract, while those from the fasciculus communis descend on the inner side of this tract in the medulla and, turning outward, emerge below, forming another of the components of this root. This is in

contrast to the second root where these fibres passed through the upper part of the ascending Trigemini. In the Golgi preparation, from which the figure is taken, a considerable number of the fibres from the fasciculus communis are impregnated and appear as straight, delicate fibres proceeding parallel with each other and forming a compact bundle. Carmine preparations show the same characteristic appearances for the fibres from this source, and Weigert preparations of the frog's brain show that here these fibres, proceeding in a similar manner, are fine and delicately sheathed. In the Golgi preparation only a small proportion of the fibres composing the bundle from the ascending Trigemini is impregnated, and these exhibit a marked contrast to those from the fasciculus communis. They are coarser, more varicose, and have a more irregular, sinuous course. The bulk of this latter bundle issues slightly cephalad of the bundle from the fasciculus communis. In addition to these two components there is a more ventral motor rootlet, similar to that emerging with the second root. It is a possibility that some ascending Trigemini fibres also pass out with the second root, but this is not certain.

The *fourth root* is indicated in Fig. 35. It is separated by a well-marked interval from the third root. Here, also, the fibres from the fasciculus communis curve down, around, and below the ascending Trigemini in several compact bundles. No fibres emerge from the ascending Trigemini, and there is present here, also, a ventral rootlet. As can be seen in the figures, the bundles from the fasciculus communis entering these roots diminish in bulk as we proceed caudad, *i.e.*, those entering the caudal roots are smaller. Emerging with the ventral rootlet of the fourth root, and further caudad than the other bundles, is sometimes to be seen still another very minute bundle from the fasciculus communis.

The *fifth root*, emerging some distance caudad of the fourth root, seems to derive its fibres from one source only. Its fibres can be traced caudad in the medulla some distance until lost among the longitudinal fibres of the lateral region of the medulla. This is the bundle which Osborn has identified, though erroneously as we shall see, with the fasciculus solitarius,

and has traced it caudad into the lateral columns of the cord. According to Osborn it contributes, in *Cryptobranchus* (45), to at least two of the Vagus roots.

This root is very probably motor, but would appear to be quite different in character from the ventral motor rootlets mentioned above.

A further description of the fasciculus communis will be found in another place in this paper.

All these roots now enter the vago-glossopharyngeal ganglionic complex. The general shape of this complex is indicated in the chart. It curves around the posterior end of the auditory capsule, as though pushed back by the extension of the latter, a peculiarity probably due to the very anterior position of the gill clefts and other parts relatively to the auditory capsule. The ganglion attached to its inner portion posteriorly is the sympathetic ganglion. It is drawn too large in the chart.

It is not possible to trace the exact relations of the various roots through this complex; yet, the composition of the latter can, I think, be determined in a general way, and sufficiently to throw considerable light upon its morphology.

In the first place, it appears that this complex falls into three main divisions which are indicated by the shading in the chart. The most proximal ganglionic division is connected with the nerves before they pass around the hinder apex of the auditory capsule. Immediately beyond this lies another ganglion, and still further along, and quite upon the outside of the auditory capsule, the third ganglion. It will be necessary to describe the various branches of the IX + X before discussing the character of these three ganglia.

The *first root*, on account of its large fibres, can be readily followed, and its destination may be finally treated here. Just before entering the vago-glossopharyngeal ganglionic complex it divides. The dorsal division (1), remaining in the dorsal part of the ganglion, curves around the auditory capsule and separates. The ventral division (5) becomes separated from the dorsal by an intervening bundle of fibres. It remains longer in connection with other fibres of the IX + X, pro-

ceeding cephalad for a distance after rounding the auditory capsule, and then finally separates.

These two branches arising thus from the first root are the lateral line nerves supplying the lateral line sense organs of the body; (1) soon subdivides.

Besides these principal branches, a small bundle of fibres separates from the ventral division of the first root while still in the vago-glossopharyngeal complex. It proceeds cephalad in the upper inner part of the IX + X trunk, separating as a twig (8) at 897. It will be again treated in its proper place below.

The ganglionated portion of this nerve appears to be in the region of its forking, thus forming a part of the inner of the three divisions of the vago-glossopharyngeal ganglionic complex, though sometimes a few scattered ganglion cells are found farther out along its course.

The remaining branches of the IX + X will now be described in the order in which they separate from the ganglia, beginning with those most caudal. The *first branch* is the dorsal division of the bundle from the first root (1), as described above.

The *second branch* (2) is the bundle interposed between the two divisions of the first root. It consists of rather small or medium fibres with a few large ones intermingled; in other words, it resembles those branches of the Trigemini and the Hyomandibularis, which we have seen to have a general cutaneous distribution. It proceeds cephalad close to the auditory capsule to 956, where it turns ecto-dorsad, and is distributed to the skin dorsad and mesad of the caudal extremity of the auditory capsule. It has no connection with lateral sense organs. This branch is evidently the same as the one in the frog, known as the *R. cutaneus dorsalis*. Thus the statement made in Ecker's *Anatomy of the Frog* (p. 174), that this branch is the persistent portion of the *R. lateralis vagi* in the tadpole, is erroneous. The supposition of Stannius and Fürbringer, there referred to, that it is the homologue of the *R. auricularis*, is evidently correct — if by this it is meant that the *Rr. auricularis* and *cutaneus dorsalis* in the frog are similar branches. If it is meant that this branch is the homologue of

the *R. auricularis vagi* of higher forms, this is also correct. But this branch is not a part of the lateral line system.

Along this portion of the main trunk, the ganglion cells, which are nearly or entirely absent from that part of the trunk lying outside the apex of the auditory capsule (*i.e.*, separated by the tip of the capsule from the inner part in transverse sections), increase in number, forming the second ganglion mentioned. This ganglion, however, is confined to the outer part of the trunk, and there is a large bundle of non-ganglionated fibres running along just inside it and close to the auditory capsule.

The *third branch* (3) separates from this outer ganglionated part of the trunk (931-949) and proceeds ventrad, sloping mesad and caudad (Pl. XI, Fig. 40). It contains ganglion cells along its course, and finally, on a level with the oesophagus, contains a considerable group of ganglion cells, meriting the name ganglion. The fibres of this branch are principally fine and many of them impregnate in Golgi preparations, as is shown in the figure. Where the branch separates from the main trunk, a small twig is given off from its dorsal side, as shown in the figure, which apparently contains mixed fibres, some of which innervate a blood vessel proceeding dorsad here (cutaneous artery?) and some of which possibly innervate a muscle just outside the auditory capsule.

Three principal branches proceed usually from the group of ganglion cells. They proceed caudad, some giving off branches to the oesophagus. Others were not traced completely, but they are probably pulmonary or gastric branches. One branch soon curves mesad and, proceeding cephalad, reaches the heart, which it innervates. It is ganglionated on the auriculo-ventricular septum (Bidder's ganglion). From this ganglion the nerve passes down along the ventricle, giving off innumerable branches, which penetrate to every portion. The general appearance of this plexus is indicated in Pl. IX, Fig. 14. As this shows, there are also numerous fibres innervating the walls of the auricles.

The third branch is the *R. visceralis*.

In addition to these branches there are other minute twigs

from the clump of ganglion cells mentioned above. One branch, containing coarse and fine fibres, proceeds cephalad and supplies, in part at least, one of the Mm. levatores arcuum branchialium.

The *fourth branch* ($4+5$) from the main trunk emerges close to the third, immediately cephalad of it. This really consists of two branches, and immediately divides. The dorsal division (5) is the ventral division of the first root, which has been already described, and which separates here (929) to form one of the Rr. laterales. The other division (4) consists of rather large and small fibres intermingled. It immediately separates from the R. lateralis, proceeds directly ventrad a short distance and then bends cephalad. It divides at 938; the outer, smaller division ($4a$), containing most of the coarse fibres, soon enters the M. levator (?) lying just outside. The remainder ($4b$) proceeds cephalad giving off a minute twig, which appears to enter another M. levator (898). At 888 it divides again, the outer subdivision ($4b_1$) apparently containing about all the remaining coarse fibres. The inner subdivision ($4b_2$), the *R. laryngeus*, turns mesad (882) and enters the laryngeal muscles ($860 \pm$). The outer subdivision ($4b_1$) has a complicated and peculiar course. It proceeds directly cephalad, coming to lie immediately above the heart. It now, still proceeding cephalad, slips ventrad close beside the heart, and at 720 appears to give off a fibre or two to a longitudinal muscle near it and near the heart. It divides at 700, and the outer division at $550 \pm$ enters a longitudinal muscle lying above it here. At 627 some fibres which previously separated from the inner division go over to the outer, which divides and innervates the muscle just mentioned. The remainder of the inner division finally unites with the Hypoglossus, and cannot be followed further as a separate bundle. None but motor branches were observed from the Hypoglossus; and from this fact, as well as from the distribution of the outer division of this branch, that portion uniting with the Hypoglossus may be inferred to have a motor distribution. This muscle, or muscles, innervated, certainly correspond, in part at least, to Schulze's M. diaphragmato-branchialis medialis.

In Ecker's *Anatomy of the Frog*, p. 183, it is said that "Hoffmann describes a communicating branch of the Hypoglossus to the 'pneumogastric nerve,' which the translator has not been able to discover and which no other observer has mentioned." It is quite probable that this is the corresponding branch in the tadpole, thus partly verifying Hoffmann's observation. In a tadpole examined in which the metamorphosis had begun this branch did not unite with the Hypoglossus. It appeared here to innervate possibly the *M. interbranchialis* also. Whether it was concerned with the innervation of any of the *Mm. marginales* could not be certainly determined. Branches 3, 4, 5, 6, and 7 are given off practically together, and from them some coarse fibres separate, forming the *R. accessorius* to a capito-scapular muscle probably representing the *M. cucularis* (= 4 a?).

At the point of separation at 929 of the branch just described, the trunk of the IX + X consists of two portions. The outer of these is mainly fine-fibred, but with some coarse fibres also. It is still ganglionated, the ganglion cells belonging apparently to the fine fibres. The inner portion is described below. The outer ganglionated portion now bends ventrad, separating from the inner, and divides (910) (6 and 7). It here also loses its ganglion cells. The ventral of the two divisions (6) gives off two or three fine-fibred twigs, which supply the epithelium of the outer angle of the pharynx in this region and some distance cephalad. One of these twigs (6 b) proceeds cephalad and at 759 gives off a twig which contains a few ganglion cells and supplies the mucous membrane of the pharynx ventral to it. At 757 the nerve contains a clump of ganglion cells, and here divides. The inner part (6 b₁) passes mesad to the mucous membrane of the roof of the pharynx. The outer part (6 b₂) supplies the membrane of the roof of the gill cavity, which opens into the pharynx here. The remainder proceeds caudad to 919, around one of the *Mm. levatores*, and bends cephalad under the latter, giving off a small twig (6 c) proceeding ectad, which could not be traced further. This twig takes from the nerve the few coarse fibres it contained. There is reason to believe (see p. 143) that this twig has finally a cutane-

ous distribution. The main nerve (6) now proceeds cephalad under one of the gill bars, a blood-vessel lying below it. At 822 it sends a branch around outside the cartilage to its dorsal side. At 788 the remainder assumes the same position. Its distribution is partly to the gill-raker attached to the dorsal side of the cartilage. Ganglion cells appear in it in places along its course, especially as it nears the point where the gill membrane is merged with the pharyngeal mucous membrane. Here (690 \pm) the nerve lies in the floor of the pharynx, near the heart. It breaks up here, part of it turning caudad, and all of it being distributed, as far as could be observed, to the pharyngeal epithelium. It is obviously a *R. branchialis*.

Returning to 910, the dorsal of the two divisions (7) receives a short, reënforcing branch from the other, and proceeds cephalad and ectad as far as 840 \pm , where it proceeds ventrad along the dorsal and outer side of the blood-vessel and turns caudad, still proceeding ventrad and thus rounding one of the Mm. levatores, where it again turns cephalad. This nerve contains two very large and prominent fibres. As it turns (870 \pm) it gives off a twig (7 α) containing these two coarse fibres, together with a number of smaller ones. It is evident that this twig resembles the one (6 c) given off from the preceding nerve, which could not be traced. This twig is larger, however, and can be traced to its distribution. It divides just subsequently to its separation. One portion (7 α_1), containing one of the coarse fibres, proceeds cephalad around the angle of the branchial cavity, through a thin muscle in the body wall, and thus becomes subcutaneous. It does not appear to give any fibres to the muscle, and is distributed to the skin. This division of the twig is thus probably of the "general cutaneous" type. The other division (7 α_2) rounds the angle of the body cavity at 777, and is in every way similar to 7 α_1 . After giving off this twig, the main nerve (7) passes under one of the gill bars — the one next to the one under which the last-described branch ran. At 807 a fibre or two comes off, which proceeds ventrad, but could be traced only a few sections. At 767 a small branch separates, which could be traced cephalad some distance, running near a blood vessel. At 613 the nerve,

having in the meanwhile, like the other, passed around to the dorsal side of the cartilage and lying in the gill-raker attached, bends mesad, nears the place of transition of the gill membrane into pharyngeal epithelium, and rounds the extremity of a small, pouch-like evagination, or pocket, of the pharyngeal cavity, which projects anteriorly. The distribution of this nerve seems to be in every respect similar to the preceding one. It also contains ganglion cells. This branch is also obviously a *R. branchialis*.

At 923, at about the point of separation of the last two branches, the remainder of the trunk of the IX + X becomes ganglionated. A wedge-shaped bundle in the outer central portion of this part of the trunk consists exclusively, or nearly so, of fine fibres, the other portions contain mixed fibres. The ganglion cells are small and seem to be confined especially to the fine-fibred portion.

At 897 a small twig (δ) separates from the inner side of the trunk. It is coarse-fibred, and proceeds ectad and cephalad from under the edge of the auditory capsule and then turns directly cephalad in the lowest, loose connective tissue, layer of the skin. At 838 it divides. The two divisions proceed cephalad and probably supply lateral sense organs, though only traceable to their vicinity. Its fibres resemble those of the other lateral line branches, and it is the portion which has been described above (p. 139) as separating from the ventral division of the lateral line bundle while in the vago-glossopharyngeal complex. It is this small branch which has been described in fishes by several observers as arising from the IX and innervating a canal organ, and which has been confused with the *R. cutaneus dorsalis* (*auricularis vagi*) in the frog (*vide supra*). It may be called the *R. supratemporalis*.

At 854 the main trunk of the IX + X falls into two divisions. The outer of these divisions (g) comprises the exclusively fine-fibred bundle and a portion of the mixed fibres, viz., that portion on the outer side of the fine fibres. The inner division (10) comprises the remainder of the mixed fibres. The ganglion cells still found in the outer division are in the fine fibred portion.

The outer division (*9*) soon subdivides ($852 \pm$) into a lower or ventral subdivision (*9a*) composed of a portion of the fine fibres, and an upper, or dorsal, and larger subdivision (*9b*) composed of the remainder of the fine fibres and the mixed fibres. The lower subdivision (*9a*) proceeds along the roof of the pharynx a considerable distance, giving fibres to it and to some portions of the filtering apparatus. It finally turns meso-ventrad, runs inward along the floor of the pharynx and supplies the epithelium of the roof of the gill cavity. This branch is obviously similar to those described previously (*6b*, etc.) as innervating other portions of the pharynx. It is one of the *Rr. pharyngei*.

The upper subdivision (*9b*) (852) proceeds cephalad and ectad, supplying fibres to the *M. levator arcus branchialis* in its vicinity, and comes to lie outside the outer angle of the pharynx. At 654 it turns ventrad, passing through a thin muscle lying in the body wall, and divides (*9b₁* and *9b₂*).

Before this division occurs, the fibres in the upper subdivision (*9b*) were so arranged that the mixed fibres occupied the outer and the fine fibres the inner side of the nerve. When the division takes place, however, the anterior, larger branch (*9b₁*) receives the fine fibres and also a bundle of coarse fibres (with sheaths deeply stained), and the posterior branch (*9b₂*) receives mixed fibres.

The anterior, larger branch (*9b₁*) proceeds cephalad and ventrad along near the inner side of the body wall, *i.e.*, around outside the gill cavity. It then bends cephalad, and again mesad, around under the gill cavity. During this part of its course the coarse fibres are very plainly seen occupying its ventral side. The fine fibres constitute the bulk of the nerve. Finally, as the nerve proceeds mesad the majority of the coarse fibres separate out ($530 \pm$). As they separate out (*9b_{1a}*) there is a small group of ganglion cells apparently on the coarse-fibred bundle. Some of the fibres of this branch innervate a muscle which appears to correspond with Schulze's *M. ceratohyobranchialis*. Other fibres seem to merely scatter in the loose connective tissue and could not be traced. This apparent peculiarity was observed in more than one specimen.

A few coarse fibres remaining in the main nerve soon separate and appear to innervate a minute muscle lying near the one previously described, and which might be identified as Schulze's *M. basihyobranchialis*. The main nerve (*9b*₁) then proceeds cephalad in the floor of the pharynx, giving off twigs to its epithelium. It finally terminates at the rudiment of the tongue, taking at this point a sharp little turn mesad. This is, of course, the *R. lingualis glossopharyngei*.

Returning to 654, the posterior division (*9b*₂), consisting of mixed fibres, proceeds caudad and ventrad along the inner side of the body wall until at 740 ± it curves, first ventrad and then dorsad and ectad (∪-shaped), around the angle of a fold of the body cavity wall and thus becomes subcutaneous. The bulk of it proceeds cephalad and lies, at 698 ±, near a lateral line branch. It breaks up to supply the skin of this region and ventral to this. It is at once apparent that this posterior branch is similar to the two twigs (*6c* and *7a*) given off by the two previously described Vagus branches, one of which could be traced to a cutaneous distribution.

Returning to 854, the other inner division of mixed fibres (*10*) is the *R. communicans ad facialem* to the *R. hyomandibularis*. Its final distribution is described in connection with the latter, and it has there been found to be a general cutaneous nerve.

Now that the branches have been described, their relations to the ganglia may be made more intelligible. As described above, the most distal ganglion (*C*), from which issues the *R. lingualis*, belongs to the fine fibred portion of the nerve at this point. The larger mixed fibres which pass off into the *R. communicans ad facialem* and the cutaneous branches do not appear to be ganglionated here. The majority of them form a bundle on the inner side of the nerve next to the auditory capsule. As we pass further proximad along the nerve trunk, the fine fibres cease to be ganglionated and form the wedge-shaped bundle mentioned, occupying the outer central part of the trunk.

The proximal portion of this ganglion is slightly overlapped externally by the second ganglion (*B*). From the distal apex

of this ganglion emerges the Rr. branchiales, to whose fine fibres this part of the ganglion belongs. This is apparently to some extent distinct from the proximal part (B_1) of the second ganglion, being partially separated from the latter by the lateral nerve issuing here. From this proximal part of the second ganglion (B_1) the R. visceralis emerges. Along the inner side of this ganglion also proceed the non-ganglionated fibres mentioned above, together with additional fibres from the cutaneous and motor branches which have in the interval been given off. The fine-fibred R. visceralis must be in part, and perhaps is mostly motor, yet it seems to be ganglionated. Whether it is entirely ganglionated, however, could not be determined.

The innermost or proximal ganglion (A) belongs chiefly to the cutaneous branches whose fibres, we have seen, pass by mesad of the two distal ganglia, and also to the N. lateralis. The former are in all probability derived from the ascending Trigemini. It is very probable, however, that some of the fine fibres, especially, perhaps, those of the R. visceralis, are also ganglionated here.

These ganglia may be designated A , B , and C , as already indicated, A being the most proximal one. B may be subdivided into B_1 and B_2 .

In the older tadpole above referred to (p. 142), the trunk of the IX + X, after giving off 1 and just as it gives off 2, divides. From the upper and outer division are given off 3, 4, 5, 6, and 7, from the lower and inner division are given off 9 and 10. As in the other tadpoles, this latter division is ganglionated further distally (ganglion C). The Rr. branchiales are reduced and the R. visceralis has apparently increased.

It will be necessary now to describe some points in the finer terminations of certain of these branches. The velar folds mentioned above, a small portion of the filter apparatus, indifferent epithelium, and also some taste bulbs, both on the roof of the pharynx and on its floor in the region of the gill openings, are innervated by the pharyngeal branches described above. The innervation of the taste bulbs is as described above under the R. palatinus VII. That of the glands, which are so plentiful here, requires further notice on account of its

remarkable richness. It is not unlikely that the appearances may be exaggerated by inequalities of impregnation, but in Golgi preparations all of this epithelium presents pictures remarkably rich in nerve fibres. We have seen above (p. 124) that the glands in this region are so numerous as to be practically continuous and not separated by indifferent epithelium. In such places the superficial nerve plexus belonging to these glands and described above (pp. 125, 126), naturally is also continuous, and there is consequently a dense plexus extending throughout the superficial part of this epithelium. Its general appearance is indicated in Pl. IX, Fig. 23, and is still more dense in preparations from a larger and probably older specimen.

The greater part of the filtering apparatus, and also the gills, are innervated by the Rr. branchiales. There is a great difference in my preparations between the innervation of these two structures. While in the former are demonstrated a great number of fibres, very few are shown in the gills proper. These latter fibres are very delicate and cannot usually be followed very far into the gills. They proceed along with the blood vessels and seem to be vaso-motor. It is not improbable that the fibres in gills do not impregnate readily, and that the supply is greater than the preparations would indicate.

Into the filtering apparatus bundles of fibres pass upward from the Rr. branchiales. These pass along in the loose connective tissue in the interior of these structures, send fibres into the smaller subdivisions, or side-pockets, and fill these with a snarl or tangle of fibres (Pl. VIII, Fig. 9). Endings are frequently seen on these fibres in the shape of small granules, or sometimes, apparently, several granules. Sometimes the two granular terminations of two fibres seem to meet each other (\times , Fig. 9). Very often these terminations are in the very outer surface of the filament of the filtering apparatus. The fibres do not usually, at any rate, anastomose.

These gill filters, together with the glandular epithelium described above, evidently form an important apparatus, physiologically. There would seem to be a physiological similarity between these glands and those on the endostyle of *Amphioxus*.

ANALYTICAL SUMMARY OF THE GLOSSOPHARYNGEUS AND VAGUS.

| ROOTS. | SOURCE. | GANGLION. | BRANCHES. | DISTRIBUTION. |
|--------|-----------------------|---|--|--|
| 1st | Tuberculum acousticum | A | { Rr. laterales (1 and 5) R. supratemporalis (8) | Lateral lines. Lateral line organs near ear. |
| 2d | Fasciculus communis | C | { R. lingualis (9 b) R. pharyngeus (9 a) | Tongue and pharynx. Pharynx. |
| | Motor nucleus . . . | Non-gang. . . | R. not named | Mm. levator arcuum branchialium, ceratohybranchialis and basihybranchialis? |
| 3d | Ascending trigeminus | A | { R. auricularis (2) Rr. cutanei (6 c, 7 a, and 9 b ₂) R. communicans ad VII (10) | Skin near ear. Skin of operculum. Skin at base of operculum. |
| | Fasciculus communis | { B ₂ B ₁ and A? | { Rr. branchiales } (6 and 7) Rr. pharyngei } { Rr. gastricus, pulmonaris and cardiacus. R. visceralis (3) | { Gills and filter apparatus. Pharynx. |
| | Motor nucleus . . . | Non-gang. . . | Rr. not named | Oesophagus, heart, lungs, etc. Prob. Mm. levatores arcuum branchialium and (from Rr. branchiales) marginales. |
| 4th | Fasciculus communis | B ₂ , B ₁ or A? . . | { Rr. branchiales } (6 and 7) Rr. pharyngei } | { Gills and filter apparatus. Pharynx. |
| | Motor nucleus . . . | Non-gang. . . | R. not named | Prob. Mm. lev. arc. branch. and marginales. |
| 5th | Ascending lateral . . | Non-gang. . . | R. laryngeus + ? (4) | Laryngeal muscles + M. diaphragmato-branchialis medialis? |

The preceding table of the roots and branches of the IX + X is given for clearness. It is imperfect in some particulars and a number of points are assumed. The motor fibres especially require further investigation, nor can the different roots be followed through separately, if, indeed, they do remain separate, which is not probable. Yet it will give, I believe, some insight into the composition of this complex.

4. *The Sympathetic.*

The general relations of the *sympathetic* to the vago-glossopharyngeal and Gasserian ganglia may be quite briefly expressed. From the ganglion cervicale sympathicum (see chart) one or more branches pass to the vago-glossopharyngeal ganglia. One twig especially can be traced curving around the inner and then the dorsal side of the caudal apex of the inner ganglion. The greater part of this twig unites with the R. auricularis. Other minute twigs may be seen ramifying around the ganglia and appear to pass off, in part, to the various branches. I have not observed any especial supply to the R. visceralis and the latter must be regarded as composed very largely of fibres from the Vagus. The same probably applies to its subdivision, the R. cardiacus.

From the various parts of the IX + X, especially the fine-fibred portions of the trunk and branches, are often seen vaso-motor fibres given off to blood vessels. Along these nerves also, especially the R. visceralis and Rr. pharyngei, particularly among their twigs, are found ganglion cells. These cells usually have a bipolar appearance, but when examined closely one or both of their processes show here and there a splitting indicating a multiple character (Pl. XI, Figs. 41, 42, and 43). The process or processes from one end may supply a blood vessel, as is beautifully shown in Pl. VIII, Fig. 13. At other times it apparently innervates epithelium, though this is not so certain. Whether all these vaso-motor fibres are derived from the sympathetic, cannot be determined, but I regard it highly probable that they are not, and that a proportion of them are from the Vago-glossopharyngeus. This is what would be

expected from the innervation of the heart. The ganglia in the latter might be regarded as collections of the ganglion cells just described.

The remainder of the sympathetic passes within the cranium through the vago-glossopharyngeal exit and forwards to the Gasserian ganglion. Its destination here has already been partly described. It supplies the various branches from this ganglion, as already mentioned by De Watteville (66). The lateral line branches appear to be, at times, unsupplied. This deficiency is possibly provided for by the smaller trigeminal branches, as described already (p. 119), from which, apparently, sympathetic fibres pass over into the lateral line branches.

Vaso-motor fibres can be seen at times coming from the trigeminal branches, and they are derived from the fine, probably non-medullated fibres, which are impregnated by Golgi's method, and which are seen in all the trigeminal branches. I have not observed any ganglion cells in connection with these fibres. Whether they are partly or wholly from the sympathetic, I have not determined.

5. *Recapitulation of Nerve Components.*

From the above description it is evident that we have in each nerve the following components, distinguishable by the nature of their fibres, their peripheral distribution and their internal origin:

Trigeminus. — (a) What may be termed *general cutaneous* fibres, *i.e.*, those supplying the skin exclusive of the specialized lateral line sense organs. The majority of these fibres are small but there are also among them a number of fibres of medium size as well as some coarse fibres. The bulk of this component is derived from the ascending trigeminal tract which is a continuation of the dorsal columns of the spinal cord. The ganglion (or ganglia) of this component is the ganglion of the maxillo-mandibularis and ophthalmicus trigemini, namely, the Gasserian ganglion proper.

(b) *Motor Fibres.* — These are mainly coarse, innervate the jaw muscles supplied by the Trigeminus, and are derived from the trigeminal motor nucleus (and descending tract?).

Facialis.—(a) What may be termed *special cutaneous* fibres, *i.e.*, those innervating the specialized lateral line sense organs. These fibres are uniformly coarse and enter the dorsal part of the medulla. There are two ganglia belonging to this component. One, the ganglion of the Rr. ophthalmicus superficialis and buccalis (and oticus) facialis, has its permanent position above the Gasserian ganglion proper and in contact with it. The other, the ganglion of the R. mandibularis externus VII, is, in the tadpole, imbedded in the outer side of the Gasserian ganglion, or rather between the latter and the ganglion of the next mentioned component. In *Amblystoma*, however, this ganglion has no relation to the Gasserian ganglion and lies beneath the outer side of the auditory capsule ectad of the ganglion of the next component and of the auditory ganglion.

(b) What may be termed the *fasciculus communis* component. This innervates the anterior portion of the pharynx. This component is composed principally of very fine fibres with a number of slightly larger fibres interspersed; it is derived from Osborn's fasciculus communis. Further remarks upon this tract and its distribution will be made elsewhere. This component possesses one ganglion which is fused in the tadpole with the ventral side of the caudal portion of the Gasserian ganglion proper. In *Amblystoma*, however, this ganglion has no connection with the Gasserian and lies beneath the cephalic end of the auditory ganglion.

(c) *Motor* fibres in part similar to Trigemini (b) and in part derived directly from the posterior longitudinal fasciculus (Osborn).

Glossopharyngeus and Vagus.—(a) *General cutaneous* fibres similar to Trigemini (a) and derived from the ascending Trigemini. The ganglion of this component is a portion of the mass of ganglion cells on the trunk of the IX + X nearest the medulla (ganglion A).

(b) *Special cutaneous* fibres similar to *Facialis* (a) in every respect and passing into the Rr. laterales. The ganglion of this component lies in the dorsal part of the ganglionic mass just mentioned (*i.e.*, of ganglion A).

(c) *Fasciculus communis* fibres similar to *Facialis* (b), its distribution being to the alimentary canal and its outgrowths caudad of the area of distribution of *Facialis* (b). There seem to be at least three ganglia belonging to this component, one of these being a portion of the ganglionic mass mentioned (A) and the other two the two ganglia lying farther out on the trunk of the IX + X (ganglia B and C).

(d) *Motor* fibres probably similar to *Trigeminus* (b).

Besides the above there are other portions of the IX + X whose nature and position is not clear. One of these is the "*fasciculus solitarius*" mentioned by Osborn, but erroneously so called (*vide infra*, p. 186), and which is also present in the tadpole. It is almost certainly motor.

Rearranging the above, we have at least four components:

I. *General cutaneous*, including V (a) and IX + X (a).

II. *Special cutaneous*, or *lateral line*, including VII (a) and IX + X (b).

III. *Fasciculus communis*, including VII (b) and IX + X (c).

IV. *Motor*, to branchial muscles (including jaw), including V (b), VII (c), and IX + X (d).

It is not to be supposed that this forms an exhaustive analysis of these nerves. Reasons may easily be adduced from this research, as well as from other sources, to show that this analysis is not complete. It is carried as far as can be conveniently done with the means employed, and will form a basis for additional results in the future.

III. COMPARATIVE MORPHOLOGY OF THE COMPONENTS.

1. *General Cutaneous*.

General Cutaneous Component. — V (a) and IX + X (a). The part of the V belonging to this component is very constant apparently in the vertebrate series. We find that the *Trigeminus* has an ascending tract from the spinal cord in *Petromyzon* (Ahlborn), in *Acipenser* (Goronowitsch), in *Selachians* (Rohon), and in *Teleosts* (Mayser, Wright). Furthermore, this tract seems subject to less variation in size than other tracts and the character of its fibres is about the same. As seen

TABLE.

| | |
|---|---|
| <p>I. Preauditory. Trigeminus (sensory) proper. (Ganglion Gasseri.)</p> | <p><i>a.</i> Several small branches inside orbit. <i>b.</i> Supraorbital, but deep (R. ophthalmicus). <i>c.</i> Infraorbital (R. maxillaris). <i>d.</i> Side of head and lower jaw (R. mandibularis).</p> |
| <p>General Cutaneous (ascending Trigeminus).</p> | <p>II. Postauditory. Part of 3d (and 2d?) root of IX + X. (Ganglion in proximal part of IX + X ganglionic complex, namely ganglion A.)</p> |
| <p><i>a.</i> Branch dorsal around auditory capsule to skin (R. auricularis vagi). <i>b.</i> Several cutaneous branches separating from the branchial branches (Rr. cutanei branchiales). <i>c.</i> Communicating branch to facialis (R. communicans ad facialem).</p> | |

hereafter, some portions of the medulla oblongata undergo remarkable variations in size and development in the different types and in this respect the tract under consideration seems to stand in contrast. This is natural when it is considered that it forms the supply to the skin exclusive of the specialized cutaneous sense organs which are peculiar to certain types only, or, at least, in certain types obtain a much greater relative development.

Respecting the nature of this component there can be no question, I think, that it is similar (homodynamous) to the dorsal spinal roots, if internal origin, character of fibres and their distribution are criteria of any weight. Its fibres are a direct continuation of those in the posterior columns of the cord, which they resemble, and are distributed to the skin exclusive of any differentiations in the latter which belong especially to the branchial region. This is also Gaskell's view (23 and 24).

It follows from this that the sensory Trigemini is largely equivalent to the dorsal spinal roots, minus, possibly, their splanchnic fibres. Besides the Trigemini, however, the same component is represented in the vago-glossopharyngeal group, and that to a greater extent than is commonly supposed. The homology of what is here denominated the R. auricularis vagi has already been touched upon. The branches, or branch, which Stannius and others have homologized with the R. auricularis vagi in higher forms, appear to be the supratemporal branches to lateral-line organs. There is every reason to suppose, however, that the lateral-line system of nerves completely disappears in the higher forms, especially as the root which supplies them disappears. One of these supratemporal branches in the tadpole is described above. Consequently the homologue of the R. auricularis vagi in higher forms must be the branch in the tadpole which I have described above as the R. auricularis, which has no connection with the lateral-line system and belongs to the general cutaneous system. The question arises, then, as to what is the homologue in *lower* forms of the R. auricularis vagi of the tadpole. I may here simply say that I believe that future investigation will bring out more clearly a

system of general cutaneous branches in this region coexistent with the lateral-line nerves. I have noticed in certain descriptions that dorsal branches are described which the investigator has been unable to trace to lateral sense-organs. Furthermore, Shore (56), in his work on the vagus nerve in Selachians, has described a dorsal cutaneous branch of medium fibres.

Ewart (18) also mentions a dorsal branch from the Glosso-pharyngeus, immediately beyond its ganglion, which passes upwards through the cranium to reach the skin over the auditory region, and "which apparently does not assist in supplying either mucous canals or sensory tubes." We have, in addition to this, one or two branches which separate from the lateral nerve before its exit from the cranium to supply the aural and part of the occipital mucous-canals, and which are homologous with the minute twig from the lateral-line root in the tadpole (8). Ewart and Mitchell further state (19): "The lateralis nerve behind the first branchial cleft consists entirely of special sensory, somatic fibres; in front it seems to be accompanied by a few ordinary, sensory fibres, which reach the skin."

It is possible that the R. meningeus and R. tympanicus in human anatomy are also represented by some of these general cutaneous branches in the tadpole, the R. tympanicus possibly being represented by the R. communicans ad facialem.

A further inference may be made respecting the ganglia. It has been seen above that the ganglionated portion of these general cutaneous nerves lies in the most proximal ganglion (ganglion A). It would follow from this and from what has gone before that this ganglion, or ganglia, would represent, in part at least, the two proximal, or jugular, ganglia¹ of the IX + X. This conclusion seems to be similar to Shore's from his study of Selachians (55 and 56).

By a comparison of the tadpole with the higher vertebrates it would seem that there is a considerably larger supply of these general cutaneous fibres, relatively, in the former than in the latter. This is readily accounted for, I believe, by consider-

¹ There appears to be some confusion in the nomenclature of these ganglia in the text-books of human anatomy. It is not necessary to enter into this here, however, and there need be no confusion if it is understood that the two proximal ganglia on the IX + X are meant.

ing the region thus innervated in the tadpole. It is in general the opercular region; and when, with the loss of gills, *etc.*, it either disappears or is largely reduced, the nerve supply is correspondingly diminished. Thus in the higher forms we have here not only a disappearance of the lateral-line nerves, but a reduction of the general cutaneous nerves of this region.

The homologies of the R. communicans ad facialem present considerable difficulty. In *Petromyzon* we have a branch, described by Ahlborn and others, which runs around outside the auditory capsule and connects the VII with the IX + X. This branch, however, belongs, according to the observations of Ahlborn, Dohrn (15), and others, to the lateral-line system, and, consequently, cannot be the homologue of the R. communicans ad facialem in the tadpole and frog. This is still further brought out by the fact that while in the tadpole and frog this branch is given off by the IX + X, and passes forwards to reënforce the VII, in *Petromyzon* the reverse is what occurs, the communicating branch here being given off by the VII and passing backwards around the auditory capsule to reënforce the IX + X, forming a considerable part of the N. lateralis. Kupffer considers this nerve to be a remnant of the "epibranchial" commissure found in *Ammocoetes*. This view can be best discussed under the consideration of the III component. (See, also, p. 200.)

Goronowitsch (28) describes a communicating branch between the Facialis and the Glossopharyngeus. The nature of this branch can be also best considered later. From his description it would not contain cutaneous fibres.

In *Urodela* there are described two communicating branches between the IX + X and the VII. One of these, a fine branch, is considered to be the cephalic part of the sympathetic; while the other, stouter branch, does not seem to be always present (Fischer, Hoffmann). Respecting the latter, Fischer (20) remarks that it might pass into either the cutaneous or muscular twigs of the facial branch with which it unites; and, further, according to Volkmann's researches (64), it contains no motor fibres. This agrees with the results above obtained, and this branch is probably the homologue of the R. communicans

ad facialem of the tadpole. Its manner of union with the VII is also similar to that in the tadpole. A further study of this nerve in these forms is desirable, especially as it is stated that this branch does not exist in *Menobranchus*; and yet the R. jugularis has some fine cutaneous twigs. Naturally, Fischer does not distinguish between the cutaneous nerves belonging to this component and those belonging to that next described. From what has been shown, however, there is reason to believe that the cutaneous fibres accompanying the motor branch to the M. mylohyoideus posterior belong to the former, *i.e.*, to the general cutaneous component.

2. *Special Cutaneous or Lateral Line System.*

a. Comparison with other Amphibia.—The second component has been designated, in contradistinction to the preceding, the “*special cutaneous*” component. It includes VII (*a*) and IX + X (*b*). Inasmuch as it is distributed to the lateral sense organs of head and trunk it may also be called the ‘lateral component.’

This component is in many respects a remarkable one. Its internal origin or, rather, termination is in certain tracts (and nuclei, Osborn) immediately dorsad to those constituting the origin of the Auditory. These tracts do not seem to be in any way directly continuous with spinal cord tracts as is the case with the ascending Trigemini. The origin of this component composes, apparently, then, a structure peculiar to the medulla oblongata. The fibres are large and present considerable uniformity in size. This component has evidently a constant and definite distribution, *viz.*, to the lines of sense organs ranged along the head and trunk of the tadpole. The arrangement of these branches and their ganglia has already been described; one point may be here added, namely, that the ganglion cells are not very numerous and do not usually seem to produce any strongly marked ganglionic swelling. There are not, however, very many fibres in these nerves in the tadpole, a fact which accounts for the peculiarity above mentioned. Besides this, its ganglion cells are possibly bipolar (comp. Stannius, 57).

TABLE.

| | | | |
|----------------------------|---|--|--|
| <p>Lateral line system</p> | <p>I. Preauditory. (Dorsal root VII.)</p> | <p>1. Dorsal division proceeding above Trigemini (ganglion above Gasserian ganglion).</p> | <p>(a) Several small twigs inside orbit (R. oticus, etc.). (b) Supraorbital (R. ophthalmicus superficialis VII). (c) Infraorbital (R. buccalis VII).</p> |
| | <p>[II. Auditory.]</p> | <p>2. Ventral division joining motor and fasciculus communis roots of VII, and thus forming Truncus hyomandibularis. (Ganglion on this trunk and fused with ventral part of Gasserian ganglion.)</p> | <p>R. mandibularis externus — which divides into several branches (see chart) on lower jaw and side of head.</p> |
| | <p>III. Postauditory. Most anterior (cephalic) and dorsal root of IX + X.</p> | <p>Lateral nerve ganglion in dorsal and proximal part of IX + X ganglionic complex.</p> | <p>Small temporal twig and Rr. laterales.</p> |

The homologies of this system are in the main clear. The *preauditory* portion is represented in Urodela by Osborn's "*7u* and *l*," which divides after its exit, and a part of which he saw proceed forward to the Gasserian ganglion (45, p. 67, Note). This latter part (*7u*), passing to the Gasserian ganglion in Cryptobranchus, corresponds to the division in the tadpole, which passes forwards dorsad of the Trigemini and, after passing through its ganglion above the Gasserian ganglion, divides into the infraorbital R. buccalis VII and the supraorbital R. ophthalmicus superficialis VII, together with smaller branches as already described. These supra- and infraorbital branches have been observed in the tadpole by Fischer (20) and Götze (29), who calls the branch from the VII the radix accessoria. Both investigators correctly surmised their connection with the lateral line system. The ventral division to the R. hyomandibularis, however, seems to have hitherto escaped notice in the tadpole. Fischer describes only four and sometimes only three branches from the Gasserian ganglion in Urodela, and these branches include the usual Rr. ophthalmicus, maxillaris and mandibularis of the Trigemini proper. The fourth branch, in Siredon, extends directly forwards from the dorsal surface of the Gasserian ganglion, dorsad and mesad of the eye to the nasal region. It is cutaneous. This branch is obviously the R. ophthalmicus superficialis and, indeed, Fischer himself conjectures that it is the homologue of the anterior part of the lateral system found in fishes. For the R. buccalis VII and minor twigs of the lateral system we must look among smaller branches from the Gasserian ganglion not noticed especially in Fischer's description, perhaps because bound up with the Trigemini branches,—a tendency noticeable, as has been seen, in the tadpole. To properly separate these will require microscopic examination. The cause of this difference between the tadpole and urodele forms is probably found in the different arrangement of the sense organs supplied. In the tadpole they are arranged linearly, in Urodela they appear to be scattered quite irregularly over the head, though exhibiting a tendency to concentration along certain lines. It is possible that the Urodela first pass through the linear arrangement in the course of their development.

That *ventral half* of the lateral line component of the *Facialis* which apparently unites with the VIII, actually, we have seen, unites with the motor and fasciculus communis portions of the VII, thereby forming the trunk of the *Hyomandibularis*. It is distributed, we have seen, in the tadpole to lateral line organs lying along the side of the head, and also to a line proceeding around under the ventral side. Here, again, we have in *Urodela*, as is evident from Osborn's account, a similar division of the lateral component ("VII'"), but it is not certain, in all cases, exactly into what branches it finally passes, for the arrangement of the organs is, apparently, different, a fact not surprising when the anatomical differences obtaining between the tadpole and *Urodela* are considered.

In general, according to Fischer, the arrangement of the *Facialis* in *Urodela* is as follows: A reënforcing branch is sent forwards to the *Trigeminus*, as above discussed. The remainder sends off the *R. palatinus* and, further along, the *R. alveolaris*. The discussion of these branches properly comes in another place. Besides these, there are two principal branches, the *R. jugularis* and the *R. mentalis*. Either before the separation into these latter two branches or from the *R. jugularis*, twigs are given off to the *M. digastricus*. The *R. jugularis* receives a communicating branch from the IX and supplies the *M. mylohyoideus* posterior and the subjacent skin. The *R. mentalis* divides into a branch along the side of the lower jaw, and another branch more mesal. A comparison with the figures of *Amblystoma* and of the tadpole will, I think, make the homologies perfectly clear. In the tadpole the communicating branch from the IX is received before the VII divides (as is also the case in some of the *Urodela*) into the *R. hyoideus* to the *M. mylohyoideus* and adjacent skin, which = the *R. jugularis* and the *R. mandibularis externus*, which = the *R. mentalis*. Consequently the *R. mentalis* is the branch belonging to the lateral line system. This homology must be taken with the provision that other fibres, such as general cutaneous fibres from the communicating branch from the IX, may also compose a part of the *R. mentalis*. A partial mingling of this kind we have found exists in the tadpole. Only a

microscopical examination of serial sections, probably, can determine such a point.

It is evident from the above that Osborn was mistaken in his conjecture that the lower of the two most dorsal Facialis bundles, *i.e.*, "VII *l*," is motor. Judging from *Amblystoma* also, there seems to be no functional difference between the distributions of the two divisions of the dorsal VII. Bürckhardt (12), probably following Osborn, seems to have fallen into the same error of supposing this ventral division to be motor.

The *postauditory* part of the lateral line component, going into the IX + X and emerging as the Rr. laterales is represented by Osborn's most anterior (cephalic) root of the IX (IX 1). Its similar origin to VII *u* and *l* is brought out in his paper. Urodela resemble the tadpole in having several Rr. laterales (Fischer, Ecker). In fishes subdivisions take place further caudad.

A paper by von Plessen and Rabinovicz (48) on the cranial nerves in larvae of *Salamandra maculata* demands some notice, especially as it seems to require correction in several points which are liable to lead to confusion. The authors distinguish two ganglia belonging to the Trigemini, a "principal ganglion" and, above it, an "accessory ganglion," connected with the Facialis root by their "Radix dorsalis." From the principal ganglion are derived two main branches, (I) the R. mandibularis and (II) the R. nasalis. (I) divides into (*a*) R. communicans cum supramaxillaris, (*b*) R. supramaxillaris inf., (*c*) motor twig to the M. pterygo-temporalis, (*d*) motor twig to the masseter, (*e*) cutaneous twig to jaw angle, and (*f*) R. mentalis. (II) divides into (*a*) twig to rectus superior (?), (*b*) nasal branch, (*c*) R. palatinus, (*d*) cutaneous branch to snout. From the accessory ganglion arise (I) R. communicans c. n. faciali (from the Radix dorsalis) (II) R. frontalis, cutaneous and supraorbital, (III) R. supramax. sup., infraorbital and cutaneous, and giving off a communicating branch to the R. palatinus facialis.

Continuing, they describe the Facialis as arising with the Acusticus. While traversing the auditory capsule it gives off (I) the R. palatinus. After separating from the Acusticus it divides into (II) the R. buccalis and (III) R. hyoideo-mandi-

bularis. (II) after giving off the (*a*) R. alveolaris, termination not stated, divides into (*b*) a branch accompanying the R. hyoideo-mandibularis, cutaneous, and a (*c*) cutaneous branch along the outer side of the lower jaw. (III) receives the communicating branch from the IX and supplies the M. depressor maxillae inferioris, M. intermaxillaris posterior and skin of the lower jaw.

The following criticisms may be offered upon these results: Their "accessory ganglion" evidently corresponds to the lateral ganglion above the Gasserian ganglion, from which proceed the R. ophthalmicus superficialis VII and R. buccalis VII. So R. frontalis = R. ophthalmicus superficialis, and R. supra-maxillaris sup. = R. buccalis, excluding any trigeminal elements that may be fused with them. The communicating branch between the latter and the R. palatinus VII, should be between the R. maxillaris V and the R. palatinus. As the R. max. V seems to be reduced or absent (= their R. supramax. inf.?) and their R. supramax. sup. contains trigeminal elements (from their principal ganglion), this discrepancy may be more apparent than real. In Wiedersheim's *Grundriss* this connection is in one place, by some error, spoken of as between the R. palatinus and R. ophthalmicus profundus.

It is obvious that the branch named R. buccalis by von Plessen and Rabinovicz is misnamed. The branch so named by them corresponds to the R. mandibularis externus (= Fischer's R. mentalis, which they have overlooked in his description, or confused with the V proper), and is derived from the ventral division of the lateral root (= their R. communicans c. n. faciali).

The connection between this system of nerves, *i.e.*, R. ophthalmicus superficialis VII (R. frontalis), R. buccalis (R. supra-maxillaris), and R. mandibularis externus (R. buccalis), and the lateral line system, seems to have been entirely overlooked by the above authors, probably for the reason, above mentioned, that these organs do not form definite lines in many Urodela, but are more scattered. Their homology of the R. hyoideo-mandibularis with Fischer's R. jugularis is correct.

These authors have also overlooked the motor and fasciculus communis roots of the VII, owing to their close adhesion to

the VIII, and also the ganglion on the fasciculus communis root, which should be at the point where the R. palatinus is given off. The "ganglion buccalis" (= ganglion on ventral half of dorsal VII) of the authors, as shall be seen below, has nothing to do with the ganglion geniculi nor their R. buccalis (R. mandibularis externus) with the chorda tympani.

Another correction of their paper is to be made in connection with the IX + X. According to their text and figures, the anterior (farthest cephalad) and undoubtedly lateral line root emerges from the ganglion, not as the N. lateralis, but as the N. glossopharyngeus. I have traced this coarse-fibred anterior root in *Amblystoma* larvae through the vago-glossopharyngeal ganglionic complex until it emerges posteriorly as the N. lateralis just dorsal to and parallel with the R. visceralis (R. intestinalis), as figured also by von Plessen and Rabino-vicz. This error probably arose from these authors supposing that the most anterior root of the IX + X series must be the Glossopharyngeus.

Arnold (5) has described an interesting condition in *Pipa Americana*, where the VIII, VII, and V are fused at their origin, and the VII and V remain in continuity as far as the Gasserian ganglion. As his work was done upon young specimens, it is possible the lateral line nerves are among those described, and, in fact, the R. ophthalmicus superficialis VII is apparently there identified. What other nerves are to be identified as belonging to this system it is hardly possible to point out, especially as their fusion with the trigeminal branches is here probably carried to an extreme.

I have myself observed in members of the Hyliidae, that the separation between the roots of these nerves is quite slight.

Another difference observable between the condition of this component in the tadpole and in Urodela, as seen in *Amblystoma* and *Cryptobranchus*, is that it is relatively considerably larger in the latter type. Its final disappearance in the Anura seems to be foreshadowed in the tadpole. (An excellent instance of developing embryonic abbreviation.)

A peculiar circumstance connected with this diminution in the tadpole is the fact, already referred to, that the space

relinquished by the dorsal VII is taken up by the VIII. The dorsal VII completely disappears in adult Anura, and the interesting question arises: Does the dorsal VII really atrophy, or does it merely pass over into the VIII? This part of the VII is much more distinct from the VIII in Urodela than in the tadpole. In the former it has a distinctly separate exit; in the latter it and the VIII emerge from the medulla together, the dorsal VII soon separating, apparently as a branch from the VIII. It is certain that when the dorsal VII has disappeared its place is completely occupied by the VIII. The question, however, as to whether there is an extinction or a transference of the dorsal VII can only be answered by following the central terminations of the two nerves on through to the final disappearance of the dorsal VII.

This disappearance of the lateral line component of the VII probably invalidates homologies which have been advanced between its branches in lower forms and its supposed representatives in higher. One of these homologies (*R. auricularis*) has already been noticed, and another will be discussed below.

In connection with the question here raised arises the interesting problem of the relation of the auditory organ to the lateral line system. The idea that the auditory organ is connected genetically with this system, advanced by Mayser and later by Beard and developed so ably by Ayers, seems likely to find general acceptance. It is not intended to enter into this question in this paper, but it may be remarked that a general survey of the facts of the innervation of these two organs certainly points strongly to a close connection between them.

Incidentally, another point may here be mentioned about which there seems to have been some confusion and which here receives its solution. Osborn says (45, p. 66): "It is seen that, whatever may prove to be the peripheral distribution of the fibres of the fasciculus communis and posterior longitudinal fasciculus, whether to the 7th or 8th, two facts remain: first that the 8th arises ventral to the 7th, although a purely sensory nerve." In a footnote he states that Dr. E. C. Spitzka

"questions the determination of the upper bundles, 7 *u-l*, in Fig. 15, as parts of the Facial, on the ground that the ventral position of the Auditory reverses the usual order." It is evident that Spitzka meant by the VII the motor portion which actually is ventral to the VIII and present in nearly all vertebrates, possibly in all. Whether the "VII *u* and *l*" which disappears in the higher forms shall be also denominated the VII is largely a question of terminology.

It will now be advisable to compare the condition of these nerves in the tadpole with that in the lower vertebrates. It is not intended, however, to make here a complete analysis of these nerves in fishes especially as I have not made any extended personal investigation upon them.

b. Comparison with the Fishes.—In considering the homologue of this component among lower forms, Goronowitsch's results on *Acipenser* (28) form a convenient starting-point and may be analyzed as follows: Respecting the origin of the N. lineae lateralis, he states that it emerges from the medulla somewhat more dorsally than the Acusticus and between the exits of the Acusticus and Glossopharyngeus. It derives its fibres, which are coarse, from the "dorso-lateral" tract. The fibres form both ascending and descending systems, the latter being notably the larger, of somewhat coarser fibres and traceable to the cerebellum. The Acusticus likewise emerges from the dorso-lateral tract, dorsal to the dorsal root of the VII, "VII" here being used, as we shall see, in a narrower sense. Its principal contingents are from an ascending system in the dorso-lateral tract and a descending system traceable to the lateral part of the cerebellum. The Acusticus also receives fibres from the "durchkreuzten Fasern der hinteren Längsbündel," a part of which are interrupted by the cells of the anterior horn. Some fibres are also received from a group of large cells ventrad of the dorsal root of the VII, which latter root originates from the lobus vagi. Besides these two nerves from the dorso-lateral tract we have the coarse-fibred ventral root of "Trigeminus II" arising from this tract. This root likewise consists of contingents from ascending and descending systems, the former not traceable, the latter, not distinguishable

from the other fibres of the dorso-lateral tract, to the cerebellum. A small contingent is derived from the same group of cells that supply a portion of the Acusticus. The exit of this root is somewhat anterior and dorsal to the Facialis and, it may be added from a study of his figures, dorsal also to the Acusticus.

Besides the ventral root of Trigemini II he describes its dorsal root which has its source in the lobus trigemini. This latter structure reaches distally as far as the exit of the N. lineae lateralis and is separated from the dorso-lateral by the cerebellar "ridge." It consists of a central ganglionic mass which furnishes the principal contingent of fibres to the outer layer consisting of fibres. Relations of fibres to other cell groups which are described need not be dwelt upon here.

Still further cephalad are given off, according to Goronowitsch, the two roots of "Trigemini I." The dorsal fine-fibred root derived from an ascending system, which he has not traced, and a descending system partly to the lateral portion of the cerebellum and partly to the mid-brain. It is to be observed from the figures given that the immediate source of its fibres is ventral to the dorso-lateral tract. It is this root which we have already identified as the homologue mainly of the Trigemini major of higher forms. Its ventral coarse-fibred root is derived chiefly from the posterior longitudinal fasciculus, partly also from some cells lying near the curve made by this bundle as it emerges ("Zwischenzellen"). It will be spoken of later.

Goronowitsch, in order to overthrow Balfour's hypothesis as to primitive nerves of a mixed type and to show that Bell's law obtains also for the head, points out that the six roots of the facio-trigeminal complex may be arranged in three pairs, each having a fine-fibred dorsal and a coarse-fibred ventral root, namely:—

| | |
|--------------|--|
| FACIALIS | dorsal root from lobus vagi. ventral root from posterior longitudinal fasciculus. |
| TRIGEMINI II | dorsal root from lobus trigemini. ventral root from dorso-lateral tract. |
| TRIGEMINI I | dorsal root from ascending and descending systems. ventral root from posterior longitudinal fasciculus. |

After stating that stimulation had shown some of these roots to be motor and the difficulty of distinguishing them in embryological stages, Goronowitsch lays down the following criteria of a complete spinal nerve :—

(1) The dorsal and ventral roots have different internal origins.

(2) It arises with two roots, a fine-fibred dorsal and a coarse-fibred ventral.

(3) It possesses a ganglion.

It is further remarked by him that the distinction as to thickness of fibre is not very essential.

I may offer the following criticism. These three criteria appear to me hardly sufficient,—the dorsal root should be a ganglionated sensory (afferent) root and the ventral root a non-ganglionated motor (efferent) root. Now in respect to the *Facialis* and *Trigeminus I*, the ventral roots spring, consistently, from a bundle (or from ventral cells) which, there is every reason to believe, is efferent. This is not the case, however, with the ventral root of *Trigeminus II* derived from the dorso-lateral tract. The two other nerves arising from this tract, the *Acusticus* and *Lateralis* are *sensory* and the ventral root in question even derives a few of its fibres from the cell group likewise giving origin to a portion of the *Acusticus*.

My criticism is not especially directed against Goronowitsch's views on the relation between cranial and spinal nerves, but simply to show, from his own observations, that there is no sufficient reason for supposing that the ventral root of *Trigeminus II* is motor; and thereby to remove, in advance, a possible objection to the homology which I advocate, namely, that Goronowitsch's "ventral root of *Trigeminus II*" in *Acipenser* is the same as the one described in the tadpole as the dorsal VII, and that it and the *N. lineae lateralis* together compose the lateral line system. The character of the fibres and their similar internal origin both point to this homology and, as will be seen below, so probably does their distribution. Moreover, Stannius did not find these roots to be motor.

The above being true, the question naturally arises: what is the homologue in *Amphibia* of the *lobus trigemini*? At first

sight it would be naturally suggested that the root coming from the lobus trigemini, the dorsal root of Trigemini II, is represented in Urodela by the upper of Osborn's two Facial roots, *i.e.*, "VII *u*." This, however, I believe is not the case. As has been mentioned, both VII "*u*" and "*l*" together probably correspond to the dorsal VII in the tadpole, as is also shown by Amblystoma. Furthermore, the root from the lobus trigemini is fine-fibred, and though Osborn mentions that the fibres of VII *u* are smaller than those of VII *l*, yet the difference is slight and both are coarse-fibred. From my own observations, and from such investigations as those of Gaskell and others, I am inclined to believe that the size of the fibres is more constant, and consequently of more importance in determining homologies than would at first sight be supposed.

Before looking further for the homologue of the lobus trigemini in Amphibia, a glance will be taken at the condition in the *Teleosts*. In the teleostean medulla oblongata, as is well known, there are three greatly hypertrophied portions known usually as the lobus vagi, the lobus trigemini, and the tuberculum acusticum. Mayser (41) speaks of the common origin of the Acusticus and R. vagi lateralis, and is so much impressed by it that he terms the latter a posterior acoustic root and, further, considers the sense organs supplied by the latter as an accessory auditory organ. He also quotes Leydig, to show the similarity between the semicircular canals and their ampullae and the mucous tubes of the lateral line. Speaking of the same organs on the head he says: "Auch diese Schleimröhren haben nach Stannius nur breite Nervenfasern und zwar aus der II Wurzel des Trigemini. Die II Quintuswurzel des Stannius entspringt aus dem Tuberculum acusticum und ist bei den Cyprinoiden, wie jener Autor, p. 28, sagt, 'fast ganz verdeckt von der III Wurzel,' d. h. unserer dorsalen geknieten" (= root from lobus trigemini). From this the homology of the tuberculum acusticum with the centre in the medulla of the dorsal VII and N. lateralis of the tadpole, and with the "dorso-lateral Strang" of Goronowitsch in Acipenser is quite evident.

According to Goronowitsch, Trigemini II gives off the following branches: R. ophthalmicus superficialis, R. buccalis,

a small reënforcing branch to the R. oticus, and a stout branch reënforcing the R. hyoideus of the truncus hyoideomandibularis. This agrees with the course of the branches of the dorsal VII in the tadpole, including the stout branch to the R. hyoideus, which obviously represents the ventral half of the dorsal VII which passes ventrad and unites with the Facialis proper in the tadpole. The fact remains, however, that his Trigemini II includes the dorsal fine-fibred root from the lobus trigemini, obviously the same root as that known as the dorsal geniculate root from the lobus trigemini in Teleosts.

Wright, in his researches on *Amiurus* (72), has traced to a considerable extent, the fibres from these various roots. According to him, the bulk of the fibres of the Rr. buccalis, oticus, and ophthalmicus superficialis come from the broad-fibred tuberculum acousticum root or roots, and the motor VII is also reënforced by the latter fibres, and by others "of narrower diameter from the ganglionic complex." The bulk of the R. lateralis trigemini and ophthalmicus (profundus) are derived from the fine-fibred dorsal geniculate root. Besides these branches two strands are formed: an "infero-medial" derived principally, but not exclusively, from the dorsal geniculate root, and a "supero-lateral" derived principally from the "broad motor" (?) "fibres of the ascending and transverse root." From the former strand, and consequently principally from the dorsal geniculate root, come the Rr. palatinus and cutaneus palatinus to the mucous membrane of the roof of the mouth and gill cover; from the latter strand, and consequently from the ascending and transverse root, a branch proceeds to the musc. abductor mandibulae. These two strands are then rearranged so that both the R. maxillaris and R. mandibularis receive a portion of each strand. The latter divides into an externus and internus, the internus proceeding along the inner aspect of the jaw and ending in the mandibular barblets, teeth, and mucous membrane, as well as the intermandibular muscle. Wright is probably mistaken in speaking of the ascending and transverse root as motor. The former, ascending part, must be sensory, while the transverse is motor. That the former is not all motor may even be inferred from his own description,

inasmuch as while the R. maxillaris receives a portion of this root, yet no motor branches are mentioned in its distribution. This ascending part is the sensory Trigemini proper.

Wright's account agrees with that of Goronowitsch in some respects, but differs in others. Trigemini II, or a root from the tuberculum acusticum plus one from the lobus trigemini, gives rise to the Rr. ophthalmicus superficialis, buccalis, and oticus, and reënforces the VII. On the other hand, according to Wright, the ophthalmicus profundus is derived from the lobus trigemini; according to Goronowitsch, from the non-homologous Trigemini I. There appears to be no R. lateralis trigemini in *Acipenser*. Another important difference is that Wright assigns two Rr. palatini to the Trigemini, derived from the lobus trigemini, while Goronowitsch denies the existence of any such branches from the Trigemini, and asserts that they belong exclusively to the Facialis. This latter point will be discussed later. There should be three Rr. ophthalmici, one the profundus and the other two Rr. ophthalmici superficiales, of which one belongs to the Trigemini proper (= Trigemini I), and the other is derived from the Facialis (ventral root of Trigemini II = dorsal VII plus, in some cases at least, fibres from a root emerging from the lobus trigemini). Both Goronowitsch and Wright appear, however, to describe only two. This, together with the fact above mentioned, that the ophthalmicus profundus of one does not seem to be strictly homologous with that of the other, is difficult to account for.

It is in Stannius' splendid memoir, *Das peripherische Nervensystem der Fische* (57), that we find the most accurate account, it seems to me, of the peripheral nervous system of fishes. Stannius not merely dissected but also checked his results by stimulating the roots, and, likewise, investigated the character of the fibres composing the roots and branches.

For the N. trigemini cum nervo faciali in forms where the roots are most separated (*Pleuronectes*), Stannius describes the following roots:

1st root, stout; mixed fibres, *viz.*, mostly medium, with a number of very large fibres and a smaller number of fine fibres. Contains a motor element to the jaw muscles.

2d and 3d roots, more dorsal ("nach hintere oder obere") and originating from the Lobus medullae oblongatae, s. Lobus posterior (which also gives rise to R. lateralis nervi vagi). Fibres exclusively large, some being as large as motor fibres, e.g., those of the oculomotor, and others, in smaller number, still larger. After their exit these roots exhibit no perceptible ganglionic swelling, but closer investigation shows their fibres to be continuous with bipolar ganglion cells. These roots are non-motor. One goes over into the Trigemini, the other into the Facialis.

4th root, "entspringt abwärts von den vorigen etwas mehr aufwärts oder hinterwärts als die erste Wurzel, aus der Seite der Medulla oblongata." Fibres fine and ganglionated, but not so apparently connected with ganglion cells, which are seldom bipolar. Non-motor. A part goes over to the Trigemini, a part to the Facialis, and a part composes the bulk of the R. palatinus.

5th root, smallest, furthest caudad, issuing immediately in front of the first acoustic root. Fibres exclusively large. Motor. Goes over entirely into the Facialis.

In *Raja*, according to Stannius, we have only three roots, which closer examination resolves into four, inasmuch as roots 3 and 4 of bony fishes are in close apposition.

1. Emerges in two strands, is composed of fibres of various size and also of mixed functions, the motor fibres being in the ventral part. This root corresponds to root 1 of bony fishes.

2. Emerging close to VIII, principally from corpus restiforme. Part goes over into V and part into VII. Fibres are partly broad and partly half as broad. Some of the fibres are motor, which belong to the VII exclusively.

3. A large "hintere oder obere" root, arising above the preceding, of broad fibres and non-motor. A part mingles with 2 and part goes over into the V.

Stannius summarizes as follows:

Root I.—Motor and sensory. Fibres mixed. Belongs to the Trigemini proper. Sometimes divided.

Root II.—Non-motor, "hintere," sometimes single, sometimes divided. From corpus restiforme. Only broad fibres

connected with bipolar ganglion cells. Principally distributed to mucous canals.

Root III. — Non-motor, "hintere." Only fine fibres. Large ganglionic masses. Distributed especially to mucous membranes, skin, and touch organs, especially on the barbels.

Root IV. — Exclusively motor, arising close in front of the Acusticus and going over into the VII (coarse-fibred).

The numbers vary owing to subdivisions and fusions.

According to Stannius the Facialis proper (Hyoideo-mandibularis) divides into a posterior, or more caudal branch, the R. hyoideus, and a more anterior one, the R. mandibularis, proceeding along the lower jaw, which either gives off a branch to the mucous membrane of the mouth or subdivides into a R. mandibularis externus to the skin and mucous canals, and a R. mandibularis internus to the mucous membrane of the mouth.

In the first account (Pleuronectes), root 1, evidently, is the Trigemini proper, 2 and 3 are the lateral line roots, and 5 the motor root. Root 4, probably, is the one described by Stannius elsewhere and by other authors as arising from the lobus trigemini. The question of its homologue in Amphibia will be discussed below. There is, however, some obscurity in Stannius' account of this root. It would appear from his description to lie sometimes ventral and sometimes (*e.g.*, compare his account of Cyprinoids) dorsal to 2 and 3.

Gegenbaur (26) describes, in *Hexanchus*, the Trigemini as arising from two trunks, an anterior and a posterior one ("vordere" *Va* and "hintere" *Vb*). The former is composed of two roots with difficulty distinguishable. The posterior trunk is also composed of two roots, one, the most dorsal (*Va*) arising from a large swelling overhanging the fourth ventricle, and the other more ventral (*Vβ*) emerging from the medulla close above the Facialis, and somewhat cephalad and dorsad to the Acusticus. *Vb* proceeds above *Va* and the two enter the Gasserian ganglion, there being an intermingling of fibres. The R. ophthalmicus is derived principally, but not exclusively, from *Va*.

Jackson and Clarke (32) describe the Trigemini as arising in *Echinorhinus* from two main trunks. (1) *Va*, furthest

cephalad, more ventral, and issuing from the medulla by two roots, and (2) $V\beta$ further caudad and dorsad, and issuing from the lobus trigemini by two rootlets, one dorsal to the other. $V\beta$ proceeds above $V\alpha$ and is closely united also to the Facialis root $V\gamma$ VII, part of whose fibres issue just above the VIII.

Gegenbaur's anterior root, $V\alpha$, is the Trigeminus proper, including both the sensory element from the ascending tract and the motor root. His most dorsal root of Vb , *i.e.*, $V\alpha$, is the one derived from the lobus trigemini. $V\beta$ is, probably, the lateral line root from the tuberculum acusticum. His Facialis is the motor root plus, possibly, a root from the lobus vagi (*vide infra*, p. 193).

In Jackson and Clarke's account, $V\alpha$ = Gegenbaur's $V\alpha$ and $V\beta$ = Gegenbaur's $V\alpha$. $V\gamma$ VII is probably compound. That portion of its fibres issuing above the VIII may be derived from the tuberculum acusticum, — in fact must be unless Jackson and Clarke are mistaken in deriving *both* rootlets of $V\beta$ from the lobus trigemini — and may be also, in part, derived from the lobus vagi (compare Goronowitsch and see below, p. 193). This root must also, of course, contain motor fibres.

Marshall and Spencer (43) describe, in *Scyllium*, a R. ophthalmicus superficialis from the VII having a course closely parallel and superficial to the corresponding trigeminal branch, a buccal branch whose proximal portion forms a connecting branch with the V, and whose distal portion proceeds parallel and superficial to the maxillary branch of the V, and a posterior or hyoidean branch. With respect to the first two, the principal difference between it here and in Amphibia seems to be that the forking into the two branches, Rr. ophthalmicus superficialis and buccalis, takes place more distally in the latter, so that the common trunk of the two forms the connecting branch and contains the ganglion. Respecting the hyoidean branch, they do not seem to be aware that the cutaneous R. mandibularis externus is, in part, a nerve to mucous canals similar to the two preceding branches. They also fall into the error, the existence of which seems to have been first pointed

out by Allis in *Amia*, and later by the writer in the tadpole, of supposing that the Trigemini takes part in the innervation of the lateral sense organs of the head, an error repeated in most of the text-books.

In treating of the roots, Marshall and Spencer come to the following conclusions: The fifth nerve in the adult arises by two roots: (*a*), an anterior non-ganglionic arising by two rootlets ($V\gamma$) = "1st root of Stannius, the anterior root (Va) of Gegenbaur, the anterior inferior root (Va) of Jackson and Clarke, and the anterior root (1) of Balfour."

"(*b*) A posterior, larger ganglionic root, the ventral or secondary root ($V\beta$)" = "anterior part of the second root of Stannius, the ventral division (β) of the posterior root (*b*) of the fifth of Gegenbaur; apparently the inferior rootlet of the second root ($V\beta$), and possibly part of the third root ($V\gamma$ and VII) as well, of Jackson and Clarke; the second root (2) of the fifth of Balfour."

"The seventh nerve in the adult arises by two roots: (*a*) A dorsal root arising far up the side of the medulla, at the junction of the thickened sides and thin roof of the fourth ventricle" ("primary" root of VII, VII *a*). "This root is the third or dorsal root of Stannius; the dorsal rootlet (*a*) of the posterior trunk (*b*) of the fifth of Gegenbaur; the superior rootlet of the second root ($V\beta$) of Jackson and Clarke, and the dorsal and posterior root (3) of the fifth of Balfour."

"(*b*) A ventral root arising from the side of the medulla at a rather lower level than the posterior root of the fifth" ("secondary" root of VII, VII β). In the adult it comes to lie in close contact with the secondary root of the fifth. "This root is the posterior part of the second root of Stannius, the root of the seventh of Gegenbaur; part, or possibly the whole of the third root ($V\gamma$ and VII) of Jackson and Clarke; and the single root of the seventh of Balfour."

These investigators first showed that the R. buccalis belongs to the VII. Their homologies do not appear to me, however, to be entirely correct. Their second posterior ganglionated root (*b*) of the V is plainly the sensory root of the Trigemini proper. As such, it corresponds, together with the motor root-

lets sometimes apparently distinct and sometimes fused with the sensory portion, to Stannius's first root, to Gegenbaur's V *a*, and to Jackson and Clarke's V *a*. Their first dorsal root (*a*) of the VII is the one derived from the lobus trigemini in all probability, and is correctly homologized. Their second ventral root (*b*) of the VII must be regarded as compound, consisting of a lateral line portion, a motor, and possibly a root from the lobus vagi. Compare tadpole (see below) and Goronowitsch's account of *Acipenser*.

Ewart (18) has described these roots in *Laemargus* as follows: the ophthalmicus profundus arises by a separate root in front of the main Trigemini. The latter arises by a large root on a line with the ventral roots of the Facialis complex. Its branches are the ophthalmicus superficialis, maxillaris, and mandibularis. The Facialis includes four separate nerves: (1) Ophthalmicus superficialis, arising from the so-called trigeminal nucleus by a root dorsad and caudad of all the others. It communicates with the buccalis as it passes through the cranial walls at a higher level than the Trigemini and ophthalmicus profundus. (2) Buccalis arises behind and at a slightly higher level than the trigeminal. It is infraorbital. (3) Palatinus and hyomandibularis arise by a large root between the Trigemini and Auditory, and partly under cover of the buccalis. It receives fibres from (1), and then passes outwards with the Auditory, giving off the R. palatinus, and finally dividing into branches to muscles and to canals and ampullae not supplied by (1), nor (2), nor the R. lateralis.

Here (2) and part of (3) are lateral-line roots. (3) must be regarded as in reality compound, comprising motor and, probably, lobus vagi roots, as well as lateral-line fibres.

Burckhardt (11) has designated the most dorsal root of the Acustico-facialis "VII," and attributed to it a motor character. There can be little doubt that this is in reality the preauditory lateral-line root, and the error noted above (p. 162) has been here repeated. (See, also, page 185.)

The following table shows the probable homologies of the preauditory lateral-line roots in the descriptions of these investigators:

TABLE OF PREAUDITORY LATERAL LINE ROOTS.

| TYPE. | AUTHORITY. | ROOTS. | CENTRAL TERMINATION. |
|---------------------|------------------------|--|---|
| Cryptobranchus . | Osborn | "VII α and γ ." | |
| Acipenser . . . | Goronowitsch | "Trigeminus II," ventral root | "Dorso-lateral" tract. |
| Cyprinoids . . . | Mayser | | Tuberculum acusticum. |
| Aniurus | Wright | (Rr. buccalis, oticus and oph. sup.) | Tuberculum acusticum. |
| Pleuronectes . . | Stannius | 2d and 3d roots | Lobus medullae oblongatae s. lob. post. |
| Raja | Stannius | 2d root (?) | Corpus restiforme. |
| (Generalized type). | Stannius | 2d root | Corpus restiforme. |
| Hexanchus . . . | Gegenbaur | V β . | |
| Echinorhinus . . | Jackson and Clarke . . | part of V γ VII, probably. | |
| Scyllium | Marshall and Spencer . | part of VII δ . | |
| Laemargus . . . | Ewart | Buccalis and part of Hyomand. and Pal. | |
| Protopterus . . . | Burckhardt | VII. | |
| Larvae of Anura . | Strong | "dorsal VII" ("VII δ "). | |

Regarding the N. lateralis, we find that it has in Selachii a similar character from Shore's account also of the Vagus in the shark. According to him, the lateral-line portion of the Vagus is coarse-fibred and distinct in origin from the rest of the nerve. It also possesses its own ganglion of rather scattered ganglion cells. Its internal organ is not traced. A peculiar feature, however, is that the lateral-line part of the Vagus arises from a number of fasciculi forming the *most posterior* (i.e., caudal) of the roots of the Vagus, and somewhat *more ventral* than the others. This account differs from the exit of this root in Amphibia, Ganoids, and Teleosts, judging from the writer's observations and the papers of Goronowitsch, Mayser, Wright, and Ewart, as shown above. In all of these the R. lateralis has its exit cephalad and dorsad of the rest of the vago-glossopharyngeal roots.

I may add that in some dissections made at the Marine Biological Laboratory at Woods Holl during the summers of 1892 and 1894, upon *Galeus canis* and *Galeocerdo maculatus*, I found also that the R. lateralis invariably arose by a single root cephalad and dorsad of the other roots of the IX + X. I cannot reconcile Shore's account in this respect with those of other investigators or with my own observations.

The lateral-line nerve, I found, may be reënforced by fibres from one or both of the next two Vagus roots. What the character of these fibres is can only be surmised, and will be discussed below. Ewart and Mitchell (19) have made a similar observation, and judging from what has been quoted (p. 156), some of these at least would be general cutaneous fibres.

(c) *Résumé of the Roots in Fishes.*—In general, the arrangement of these roots and principal branches seems to be as follows :

1. The Trigemini proper, with fibres of varying sizes, which sometimes arises by two roots, in which case the one cephalad is the R. ophthalmicus profundus. Besides this latter it divides into the R. ophthalmicus superficialis, the R. maxillaris, and the R. mandibularis. It is this Trigemini proper, the central continuation of which is an ascending tract from the cord, which has also a ventral motor root added, and is constant in the main throughout the vertebrate series.

2. One or usually two broad-fibred roots arising from the tuberculum acusticum (=lobus posterior medullae oblongatae, Stannius = corpus restiforme = dorso-lateral tract, Goronowitsch). One of these passes forwards over the Trigemini proper and Gasserian ganglion and there divides into the supra-orbital R. ophthalmicus superficialis and the infraorbital R. buccalis. The other root, nearest the Acusticus, unites with a motor root, and passes into the hyoideo-mandibularis—the Facialis proper.

3. A root, most dorsal of any, from the lobus trigemini (=lobus impar). This root divides in such a way as to send fibres to both divisions of No. 2. While that part of No. 2 joining the Trigemini does not seem to necessarily form an intimate union with the latter, No. 3 seems to completely mingle with No. 2. Besides mingling with the latter, however, No. 3 in some cases forms a R. palatinus and a R. lateralis, besides other smaller branches. Often the R. ophthalmicus superficialis VII appears to be especially connected with this root.

4. A broad-fibred motor root close to the Acusticus and passing exclusively into the hyoideo-mandibularis.

5. In some cases, at least, a fine-fibred root from the lobus vagi (Goronowitsch).

The N. lateralis vagi always arises by a broad-fibred root from the tuberculum acusticum, dorsad and cephalad of the other roots of the IX + X.

I may add here that this arrangement of the roots is confirmed by my own dissections of Selachians.

It is No. 2 which innervates the lateral canals in all cases, a fact which Stannius seems to have clearly recognized, though he does not always seem to have distinguished clearly between its branches and those of the Trigemini proper. Marshall and Spencer, and Van Wijhe, I believe, were the first to make this clear. The fact that the Trigemini proper does not participate in the innervation of the lateral-line system has also been brought out by Allis (*Amia*), by Ewart (*Laemargus* and *Raja*), and by the writer (*tadpole*).

It is very evident from the above that the lateral-line system of nerves are alike in their main arrangement in all the forms

in which they have been studied and carefully distinguished. The agreement between the larval forms of Anura and the fishes is quite remarkable. The same general plan holds for both. It is owing principally to the labors of Allis and Ewart that this general plan has been made clear in fishes. As in the latter forms many variations are found, such, doubtless, will be found the case also in amphibian larvae and in Urodela, both in the arrangement of the organs and the courses and smaller ramifications of their nerve supply; but the roots and principal divisions and their arrangement will probably be found to hold good for all.

One very important difference, apparently, between the conditions in fishes and Amphibia, has emerged however; and this relates to root No. 3. The question of the fate of this root can be most conveniently considered with the next component, though in many respects it falls most properly under the component just discussed.

3. *Fasciculus Communis*.

a. Amphibia.—The third component is, as has been described, derived from the fasciculus communis.

TABLE.

| | | |
|--|---|--|
| Fasciculus communis (+ adjoining nucleus?). | Preauditory — one root (gang.) | <ul style="list-style-type: none"> R. palatinus — roof of pharynx. R. mandibularis — portion of floor of pharynx. |
| | Postauditory — several roots (gang.) | <ul style="list-style-type: none"> R. lingualis IX — part of floor of pharynx. Rr. pharyngei — pharynx and part of filtering apparatus. Rr. branchiales — gills, part of filtering apparatus and pharynx. R. visceralis — heart, lungs, oesophagus, etc. |

This remarkable tract appears in transverse sections of that part of the medulla of the tadpole just cephalad of the opening of the fourth ventricle, as an oval island, as it were, imbedded

in the ganglion cells of the central gray. It is here almost in the extreme dorsal part and quite close to the ventricle. It can be traced caudad into the spinal cord a short distance only, in the adult frog. It lies then in the posterior columns close to the median line. Judging from Weigert preparations it contains in this portion only a very few medullated fibres. According to Golgi preparations in the tadpole there are few fibres in it in this region (Pl. XI, Fig. 31). The remainder is made up apparently, of a "ground substance." The change in position and character of this structure as we advance cephalad is well shown in the Golgi preparations from which Figs. 31-39 inclusive were drawn. The tract gradually comes to lie further ventrad and laterad and also increases in size. The number of fibres in it, all fine and with a thin sheath or none, also increases very greatly as shown both by the Golgi and Weigert preparations. The maximum is reached at the level of the exit of the second root of the IX + X, which, as we have seen, derives a considerable portion of its fibres from the fasciculus. Cephalad of this, it continues as a small bundle, rather difficult to follow in the tadpole, until it passes out in one of the facial roots as described above (p. 113, see also Fig. 39). Whether a portion of it continues on still further cephalad could not be ascertained in the tadpole. According to Osborn (45), it does continue forward in *Cryptobranchus*. As seen in the figures (31-39) its course is nearly parallel with the ascending Trigeminal tract. Both undergo, on entering the medulla, a downward deflection. Consequently, if the ascending Trigeminal (and this tract?) represent morphologically, in the medulla, the posterior columns of the cord, the tracts and nuclei connected with the lateral line system are superadded structures, inasmuch as they lie dorsal to these. (Compare Ahlborn on *Petromyzon*.)

As the fasciculus communis retreats from the immediate vicinity of the ventricle or, rather, central, canal and from the mass of cells surrounding the latter in the tadpole, a number of the cells are detached with it and form a group on its inner ventral side. This group of small cells, as shown in ordinary carmine preparations, accompanies the fasciculus throughout

its course and, moreover, seems to vary in size *pari passu* with the variations in size of the fasciculus. It nearly or quite disappears cephalad of the second root of the IX+X.

The fibres of the fasciculus communis do not appear to be connected directly with nerve cells in the medulla, though, as indicated in Fig. 12, the surrounding cells frequently send their protoplasmic processes into it. As the nerves originating from it are ganglionated, such ganglia are to be considered as its nuclei of origin ("Ursprungskern," Kölliker 34), while the group of cells above described are to be considered possibly as its terminal nucleus ("Endkern," Kölliker 34). It is possible that some fibres of these roots proceed directly to the nucleus without passing into the fasciculus. From Osborn's description this would seem to be the case in *Cryptobranchus*, for besides the nuclei corresponding probably to the lateral line nuclei and the motor nuclei we have another nucleus (whose character is considered doubtful by Osborn).

The diminishing size of the fasciculus from the level of the exits of its roots on caudad in the medulla is due to the gradual loss of its fibres as they terminate freely along their route. The peculiar compound character of the fasciculus as described by Osborn is explicable when we consider that the fibres grow into the medulla by means of the various roots and there unite to form the fasciculus.

It is evident that this tract is composed exclusively or almost exclusively of *visceral* (splanchnic) fibres innervating the alimentary canal and its appendages. Whether, however, it is composed of efferent or afferent fibres or both, is not so clear. It would seem quite certain, however, that many, or most, of the fibres terminating in epithelium are to be regarded as afferent. This is true also of many of the fibres innervating the filtering apparatus. The character of the fibres so richly innervating the curious glands found in the vicinity of the opening of the gill clefts into the pharynx is not so clear. If we assume the existence of secretory fibres, it would seem certain that some of them must be of this character. We have also seen that some of the vaso-motor fibres, including those to the heart, appear to belong to this component. The fact that

these fibres have peripheral ganglia as their nuclei of origin does not necessarily disprove this, as they may in this respect resemble the sympathetic. Altogether it seems most reasonable to regard this tract together with its nucleus as composed of both afferent and efferent fibres.

This tract has been noticed by several writers, apparently, and I can hardly agree with Osborn when he speaks of it as a bundle not hitherto described if he means this to apply to Amphibia in general. Stieda, in his account of *Axolotl* (59) says: "Die stärkste oder die vorderste Vaguswurzel, welche etwa dem N. glossopharyngeus der Säuger zu vergleichen wäre, verhält sich etwas anders als die hinteren Wurzeln. Bereits ziemlich weit hinten markirt sich im oberen Abschnitt der grauen Substanz und zwar in der Kernzone eine lichte Stelle; hier sammeln sich allmählig feine Nervenfasern zu einem beträchtlichen Längsbündel, welches durch Vermittelung vieler kleiner dicht auf einander folgender Wurzelbündelchen das Mark verlässt. Das ist die Hauptwurzel des Vagus." And again in his description of the central nervous system of the frog: "Bereits in der Gegend der Uebergangsstelle des Rückenmarks in die Medulla oblongata macht sich auf Querschnitten dicht zu beiden Seiten des erweiterten Centralcanals ein rundlicher Fleck bemerkbar (Fig. 9 u. 10 k), welcher durch einige Kerne und kleine Nervenzellen eingefasst, sich von der übrigen grauen Substanz abgrenzt. Bei Untersuchung einer ganzen Reihe hinter einander liegender Querschnitte erscheinen in dieser runden Gewebsinsel anfangs spärlich, später reichlich querdurchschnittene Nervenfasern in kleinen Bündelchen. Dabei rücken die kleinen Bündelchen immer noch in der Gewebsinsel eingeschlossen allmählig der lateralen Peripherie näher, bis sie endlich derselben ganz nahe gekommen sind. Unterdess ist die scharfe Begrenzung der Bündel durch die sie begleitende Grundsubstanz verloren gegangen und statt des querdurchschnittenen Längsbündels ist auf dem nächsten Querschnitte ein starkes schräg abwärts geneigtes, abtretendes Wurzelbündel des Vagus sichtbar, dem sich ein oder zwei der früher beschriebenen Querbündel anschliessen (Fig. 11 l)." Stieda was unable to trace these

fibres to any cells. Köppen (35) seems to refer to this tract when he says : "Schon kurz vor der Eröffnung des Centralcanals, bald nachdem die letzte dorsale Wurzel das Rückenmark verlassen hat, tritt dorsal in der grauen Substanz ein Längsbündel feiner Fasern zu Tage, eingelagert in einer dichten grauen Masse, die sich kreisförmig von der übrigen abgrenzt (Substantia gelatinosa Rolando), s. Taf. I, Fig. 3 S. g. R. Diese Substanz ist nur die Fortsetzung der gelatinösen Masse, welche wir im Rückenmark, innen vor den dorsalen Wurzeln, fanden. In ihr zeigen sich nun einige Längsfasern, *ein aufsteigendes Wurzelbündel des Vagus und des Trigemini*. Es rückt allmähig aus der grauen Substanz immer mehr dem Rande zu und liegt schliesslich median von dem Seitenstrang und den Dorsalsträngen." Köppen seems to have recognized the fact that this tract is continued cephalad of the IX + X but refers it to the Trigemini instead of the Facialis. As he seems to have overlooked also the motor bundle of the VII which makes its exit with the VIII or, rather, refers it to the VIII, he is compelled to seek for the VII in the V and derives both the VII and the motor portion of the V from the motor nucleus of the V. Osborn remarks : "At this point he (Köppen) fails to distinguish between the Facial and Auditory elements, for his dorsal Auditory root, p. 9, is probably the main portion of the Facial. This error, if error it be, arises from the fact that he expects to find the facial a purely motor nerve, p. 10." Köppen must be acquitted on this point, however, for the "main portion of the Facial" referred to (Osborn's "*7 u* and *l*" = "dorsal VII" to lateral sense organs) has disappeared in the frog. Köppen's real error is in not distinguishing between the V and VII and into this error he may have been led as Osborn suggests.

Osborn gives a correct account of the exit of this bundle, but was unable to determine whether it ultimately went to the VIII or the VII. As shown above, the latter is the case. There exists a remarkable difference, however, between the exit of the fasciculus communis in Urodela, on the one hand, and in Anura and tadpoles on the other. In the former the exit of this fasciculus to the VII takes place just *dorsal* to the

VIII, while in the latter it is just *ventral* to the VIII. This change is perhaps correlated with the change in position of the Auditory, which has already been touched upon.

It may be most conveniently noted here that Burckhardt (11) describes in *Protopterus* a dorsal root belonging to the VII, partly motor, a root beneath it from the fasciculus communis, which he attributes to the VIII ("VIII₁") in his nomenclature, a second and largest root to the VIII ("VIII₂") below this, then finally and most ventral a root derived partly from the posterior longitudinal fasciculus and partly from a motor nucleus. This latter he also attributes to the VIII ("VIII_{3,4}"). Burckhardt probably follows Osborn in this assignment of these roots, which are so very similar to those in *Cryptobranchus*. In this, as we have seen already, he is mistaken. The dorsal root is the lateral line root, and is entirely sensory. The fasciculus communis root and the most ventral root should be assigned to the VII and not to the VIII. The VIII is represented entirely by the largest root ("VIII₂") between the two last mentioned.

The most interesting fact to be noticed here is that in the position of its fasciculus communis root, *Protopterus* agrees with the Urodela and differs from the tadpole, from Anura, from Acipenser, and not improbably, if the views taken on p. 193 be proved correct, from Selachii in this respect.

(b) *Higher Vertebrates*. — Before looking further at the lower vertebrates it may be well to take a glance at the higher forms. In the human medulla, as is well known, the principal sources of the IX + X are three, (1) from a motor nucleus, the nucleus ambiguus, (2) from a so-called sensory nucleus, and (3) from the fasciculus solitarius. We have also the motor nuclei of the XI, principally similar or identical with those of the IX + X.

Kölliker, in the latest edition of his *Gewebelehre*, gives a very clear and complete account of the fasciculus solitarius. It is apparent some distance caudad of the calamus scriptorius lying latero-dorsad of the sensory terminal nucleus of the IX + X, and consequently quite near the median line in the dorsal part of the cord. It gradually increases in size as it

proceeds cephalad, and also gradually becomes further removed from the median line. It finally passes out into the IX + X by some 8 or 10 rootlets, which break through the ascending trigeminal tract to make their exit. It is here this fasciculus attains its greatest dimensions. A small portion of it, however, continues cephalad, and emerges as the portio intermedia Wrisbergii of the VII. It is accompanied as far as the exits of the IX + X on its inner side by the "sensory" nucleus of the IX + X. The finer structure of this bundle is as follows: it consists of the finest fibres with gray matter intermingled; the fibres both divide and give off collaterals which encircle the nearest ganglion cells.

It is evident, from the above brief résumé of Kölliker's account, that the *fasciculus communis* corresponds with the *fasciculus solitarius* in every detail, and that the nucleus on the inner side of the former corresponds with the so-called sensory nucleus of the IX + X.

Furthermore, inasmuch as the fasciculus solitarius is continued cephalad into the portio intermedia, it is evident that the *portio intermedia* is represented in the tadpole by the fasciculus communis root of the VII, the *ganglion geniculi* by the ganglion of this root, fused in the tadpole with the ganglion Gasseri, but separate in Amblystoma, and the *chorda tympani* by that portion of the fasciculus communis which, on emerging from its ganglion, unites with the hyomandibularis VII, separates as the R. mandibularis internus, and innervates portions of the floor of the pharynx, especially that part, in the tadpole, near the site of the future tongue. The R. mandibularis internus thus corresponds, point by point, with the chorda tympani, having the same character of fibres, the same internal origin, and the same course and final termination.

That the R. palatinus would correspond, in part at least, to the R. superficialis major, would seem probable. One objection to this view, *i.e.*; that the latter is a motor nerve to the muscles of the palate, has been removed by Turner (63), who has shown that probably the muscles of the soft palate derive their motor supply from the pharyngeal plexus through the pharyngeal branch of the Vagus. Another serious objection,

however, arises from von Lenhossék's discovery (39), that the fibres of the superficialis major are not connected with the geniculate ganglion. As we shall see later, the innervation of the mouth (in the broader sense) is a complicated problem.

The homology of the R. mandibularis internus with the chorda tympani was first briefly discussed by me in the articles in the *Zoologischer* and *Anatomischer Anzeigers*. Since then Gaupp (25), reasoning from topographical relations, has come to a similar conclusion.

In Urodela the R. mandibularis internus, or chorda tympani, is represented by the R. alveolaris VII, which usually, for part of its course, proceeds in a canal in the lower jaw. Fischer was not able, apparently, to determine its distribution clearly. He mentions, however, twigs to the skin. This is probably either incorrect, or such twigs leave the proximal part of this branch and are composed of cutaneous fibres not properly belonging to the R. alveolaris. Von Plessen and Rabinovicz do not mention its final distribution.

Although anticipating somewhat, it may be here noted that Ewart describes a bundle of fibres continuous with the root of the R. palatinus and running forwards to end in the "fold of mucous membrane lying between the hyoidean and mandibular cartilages." This he regards as the homologue of the chorda tympani. Pollard (49) also regards the R. mandibularis internus as the chorda, and correctly homologizes it with the R. alveolaris in Urodela. The other view of Froriep (22), that the chorda is represented by a sensory branch to the lower jaw similar to the R. ophthalmicus superficialis and buccalis, *i.e.*, a lateral line branch is certainly not correct. In addition to the criticism made by Wiedersheim (67, p. 286), the chorda in every way corresponds to the R. mandibularis internus, as stated above, and not to the R. mandibularis externus, which innervates lateral line organs.

It is possible, however, if, as Kupffer (36) suggests, Froriep really treats of epibranchial and not lateral ganglia, that he had the correct nerve, but was mistaken in assigning it to the lateral line system.

In the IX + X the two outer ganglia on these nerves

(*B* and *C*), which belong more especially, apparently, to the fasciculus communis component, would correspond to the two outer ganglia on the IX and X in the higher vertebrates, namely, those usually designated the ganglion petrosum IX and ganglion trunci vagi. They would be partially, at least, homodynamous with the ganglion geniculi.

(*c*) *Comparison with the Fishes.*—It is evident that so important a tract as the fasciculus communis must be represented among the forms below Amphibia, and it is tolerably certain that the homologue is the *lobus vagi* of fishes.

In Goronowitsch's article on the brain and cranial nerves of *Acipenser* (28) is the following: "In der Gegend des Calamus scriptorius ist, wie gesagt, die graue Substanz des Hinterhornes breiter entfaltet. Sie hat dieselbe Struktur wie im Rückenmark. Medial vom Hinterhorne erscheint allmählich eine neue Lage von grauer Substanz. Sie besteht aus feinkörnigem Grundgewebe, in welchem viele kleine Nervenzellen eingebettet liegen (Fig. 45 L v.). Zwischen diesen Zellen verlaufen die feinsten Fasern in verschiedenen Richtungen. In proximalen Abschnitten wächst die Querschnittsoberfläche dieser Substanz. Sie verbreitet sich in dorsaler Richtung und verdrängt lateral den Hinterhornkopf. In proximalen Ebenen bildet diese graue Substanzlage einen Vorsprung im ventriculus IV. Es ist das der Lobus vagi." Goronowitsch mentions, as do other writers, the series of swellings visible in the wall of the ventricle, and caused by the lobus vagi, and says they correspond to the bundles making their exit from it. They form the dorsal source of the Vagus and Glossopharyngeus roots, exclusive of the N. lateralis, and are fine-fibred. Goronowitsch also finds that a bundle is given off from the lobus vagi to the Facialis.

In all accounts there is a remarkable agreement,—the situation in which it appears, in the vicinity of the opening of the fourth ventricle, its peculiar appearance and composition there which leads to its description as "eine lichte Stelle," "ein rundlicher Fleck," "eine runde Gewebsinsel," "ein Längsbündel feiner Fasern eingelagert in einer dichten grauen Masse," "eine neue Lage von grauer Substanz," consisting of

"feinkörnigem Grundgewebe," the appearance in it of fine fibres which form the chief supply of the IX + X,—all these peculiarities demonstrate the homology here suggested.

This tract is described by other investigators of the medulla of fishes, and there seems to be essential agreement between all their accounts, with variations respecting the number of enlargements presented by it. Rohon (52) gives a more detailed account of it in *Selachii*, and considers it as representing the summation of a number of nerve nuclei. The nerves originating from these he considers homologous to dorsal spinal roots,—a view which is discussed elsewhere.

Among the *Teleosts*, Mayser (41) gives a detailed account of the structure of the lobus vagi, which is here enormously developed. Mayser distinguishes five parts in the lobus vagi: (1) The outermost layer, comprising the bulk of the fine-fibred roots, of which there are two layers—a thicker outer of medullated fibres, and a thinner inner layer of non-medullated fibres. (2) The gelatinous substance, consisting of a dense ground-substance with numerous nerve cells interspersed. There are also solitary bundles of fine fibres here which join the first layer. (3) The secondary vagus-tract, consisting of fibres from (2) and from the "spongy substance," which is to be considered the central gray. This tract is joined by the secondary tract from the lobus trigemini, and proceeds cephalad to the higher centres. (4) The origin of the thick-fibred motor vagus-roots. (5) Ependyma.

Mayser, it may as well be added here, describes the lobus trigemini as having much the same structure as the lobus vagi, and on this ground and from the fusing of their secondary tracts he regards them as practically identical. He finally says that we have thus in *Cyprinoids* three great cranial nerve roots arising from a common nucleus, *i.e.*, the continuous substantia gelatinosa of the medulla and spinal cord, these three roots being the ascending and dorsal geniculate (lobus trigemini) Trigemini, and the sensory Vagus. The ascending and the dorsal geniculate Trigemini roots together represent the ascending Trigemini of the higher vertebrates.

Mayser apparently includes more in the lobus vagi than, for

example, Goronowitsch, namely, the nuclei of the motor roots. This, of course, does not affect the actual similarities existing. When we compare this description with the condition in the Amphibia we find them, I think, similar. Excluding the motor nuclei, we have in both cases the bundles of fine fibres, the ground substance, and nerve cells, arranged in practically the same manner. The fasciculus communis itself represents the outer layer described by Mayser, the peculiar ground-substance the second layer, and the nerve cells found by Mayser are represented in the tadpole by the group of ganglion cells accompanying the fasciculus; in *Cryptobranchus* partly, at least, by Osborn's "nucleus of small sensory cells by which the fasciculus communis is apparently reënforced" (45); and in the higher forms by the "sensory nucleus" of the IX + X, and probably by other cells in the vicinity of the fasciculus solitarius.

One great difference is apparent between this tract, or tracts, in fishes, on the one hand, and in Amphibia — especially Anura, — together with the higher vertebrates, on the other, namely, that in the fishes it is much more developed. This is easily intelligible when it is considered that it is essentially the central organ of the branchial nerve supply. Its great development in fishes is correlated with the development of the gills, and where these are in process of reduction or lost it is correspondingly reduced.

The fine-fibred branches of the postauditory portion of this component (see Table, p. 180) correspond, of course, with the similar branches constituting the fine-fibred, visceral portion of the IX + X in fishes, as distinguished by Stannius, Shore, and others. This excludes the larger-fibred branchio-motor portion. The regular arrangement of the roots, ganglia, and branches, as seen in many fishes, is, in the tadpole, mostly obscured, owing to causes noted above (p. 135). This arrangement is partly preserved, however, in the series of roots from the fasciculus communis. The ganglion of the IX (ganglion *C*) is still separate, but the ganglia of the Rr. branchiales are fused with each other and also, partly, with that of the R. visceralis. Owing not only to the forward position of the gills relative to the auditory cap-

sule, but also to the forward position of the heart and part of the viscera, the courses of these branches are likewise altered.

It will now be well to discuss the character of the *lobus trigemini*. It has already been seen that the root from this structure, where best developed, supplies fibres to the various branches of the *Facialis*, and also independently gives origin to a *R. palatinus*, *R. lateralis*, and other recurrent branches. Stannius has already stated (*v. supra*) his belief that this root was concerned largely in the innervation of the terminal buds which are found so abundantly over the surface of the head, gular plate, dorsal part of the body in some cases at least, and in the mouth and gill cavities. Wright has made similar observations as to the innervation of these organs by nerves from this root, especially around and on the barbels where the buds are so concentrated. Whether this root is devoted to the terminal buds as exclusively as those from the *tuberculum acusticum* are devoted to the canal organs, will require further investigation; that it is largely concerned with the innervation of the buds seems quite certain.

Stannius is not always clear in his description of this root. In some forms — *Spinax* and *Selachians* generally — his fine-fibred root emerges quite ventrally, on a level with the motor root of the VII. We have seen, however, that other investigators in *Selachians* have always described the root from the *lobus trigemini* as the most dorsal, and that this is its position in *Teleosts*. From this ventral fine-fibred root of Stannius is derived the *R. palatinus*. When we consider this, and also that Mayser and Wright do not describe any root of the *Facialis* derived from the *lobus vagi*; that Mayser considers the *lobus vagi* and *lobus trigemini* to be similar; that the *fasciculus communis* root of the VII in *Amphibia* sometimes emerges dorsal and sometimes ventral to the VIII (though always ventral to the lateral-line root); and that there is apparently in *Amphibia* no *lobus trigemini* root, it would seem not improbable that we were really dealing here with the same root, which simply shifted its position. On the other hand, as we have just seen, while Stannius describes this fine-fibred root as rather ventral in *Selachians*, others find in *Selachians* a dorsal root arising typic-

ally from the lobus trigemini. Finally, we have in *Acipenser* (Goronowitsch, 28), as seen above, *both* roots present, *i.e.*, a fine-fibred most dorsal root from the lobus trigemini, and another fine-fibred root emerging ventrally with the motor root of the VII.

The accounts of the R. palatinus also seem to vary. According to Stannius, it is sometimes given off by the V, sometimes by the VII, and sometimes is independent. It is sometimes reënforced by a branch from the IX (Stannius, Goronowitsch), and by Pollard (49), in *Polypterus*, is described as composed of united branches from the V, VII, and IX nerves. Goronowitsch denies the existence of any R. palatinus trigemini, and asserts that the R. palatinus belongs to the Facialis. In *Amphibia* the latter is true. It will not be possible to reconcile these apparent discrepancies until special investigations have been made upon more of the different types. It would seem quite certain that in some cases, at least, a part of the R. palatinus is derived from the lobus trigemini (Stannius, Wright), and that the significance of this lies in the existence in the mouth of numerous end buds to be innervated. It does not seem possible, however, to relegate the supply of all these buds to this source, inasmuch as we have some of them supplied by the R. lingualis IX and even, possibly, by some of the Rr. pharyngei and branchiales X in *Amphibia*. In this connection, the view of Mayser that the lobus trigemini and lobus vagi are similar structures comes again into consideration. While these two structures obviously, from the final distribution of their nerves, must be considered as largely different, yet the latter may be conceived as containing a number of fibres to end buds in the pharynx, while the lobus trigemini has been specialized off to supply the great bulk of these structures in the mouth and over the head and body.

Respecting these preauditory roots, on a careful examination of the most ventral root of the VII in *Selachians* I found this root presented the appearance of a double root. Ewart seems to have observed a similar appearance. As far as could be judged from dissections merely, the R. palatinus consists of fibres from both this double root and from the lobus trigemini

root. One part of this double root must, of course, be motor and pass into the R. hyomandibularis, the other part will, I believe, be found to arise from the lobus vagi as in *Acipenser*. I am inclined to believe that this will be found to be the condition in all fishes, namely, the coëxistence of *both* a root from the lobus vagi and one from the lobus trigemini, and, furthermore, always a R. palatinus from the former at least, the variable element in the palatine nerves being the part played by the lobus trigemini root in their formation.

Again, we have the discrepancy, which has been mentioned, with regard to the R. ophthalmicus profundus, which, according to some investigators, belongs to the Trigemini proper and according to others (*e.g.*, Wright) is derived from the lobus trigemini. It is possible that here again there is not a complete separation or specialization of the fibres to end buds in some forms (see below, however, on this point).

This branch, the ophthalmicus profundus, has been discussed by H. H. Wilder (71), who comes to the conclusion that the ophthalmic branch of the Trigemini in Amphibia represents the united ophthalmicus profundus and ophthalmicus superficialis trigemini. It seems to me that this is probably correct, but the fusion *has already taken place*, in *Salamandra maculata*, to which Wilder refers, inasmuch as the R. frontalis of Plessen and Rabinovicz is, as we have seen, the R. ophthalmicus superficialis facialis and not the R. ophthalmicus superficialis trigemini. The latter, then, does not appear to be present as an independent branch, and has already united with the ophthalmicus profundus. The ganglion of the latter has already fused, forming a part of the Gasserian ganglion, for the "Nebenganglion" is a facial ganglion.

Our view as to the fate of the ophthalmicus profundus, however, will partly depend upon whether we find it to belong to the Trigemini proper or to the lobus trigemini root. If the latter, it very possibly would have either undergone a reduction or aborted, owing to loss of end buds in the nasal region.

The results of the majority of investigators, however, certainly favor the view that the R. ophthalmicus profundus

belongs to the general cutaneous system (Ahlborn, Goronowitsch, Ewart).

I may add, in support of Wilder's view, the relation of the R. ophthalmicus trigemini, in the tadpole, to the III and IV nerves. It lies in the fork formed by the division of the III into its inferior and superior branches, and likewise comes into relation with the IV. In *Galeus canis* the R. ophthalmicus *profundus* bears precisely the same relation to the III while the R. ophthalmicus *superficialis* V bears the same relation with the IV that the single R. ophthalmicus trigemini bears to these two nerves in the tadpole.

As far as I can ascertain, the root from the lobus trigemini is probably coarse-fibred in Selachians. As the roots from the tuberculum acusticum are devoted to the canals, it would seem likely that those fibres in the lateral line nerves of the head derived from the lobus trigemini are devoted to the innervation of the ampullae. If this were true, as further research is necessary to show, the ampullae would represent the end buds of other fishes. The absence of a R. lateralis from the lobus trigemini in Selachians and the concomitant absence of such organs on the trunk is significant in this connection.

The innervation of the pit organs should be studied in this connection. If innervated by fibres from the lobus trigemini, it would appear that they have been secondarily specialized from the end buds and added to the lateral line system.

If it is true, furthermore, that the fine-fibred root from the lobus trigemini in Teleosts and Ganoids is represented by a coarse-fibred root in Selachians, we have a most interesting case bearing upon the significance of fibre-calibre. Here the principal change, apparently, in the structure innervated is a sinking below the surface and a probable increase in size.

As has been seen, there seems to be no lobus trigemini in Amphibia. This is not difficult to understand now when it is considered that the end buds are confined to the mouth in Amphibia, and, consequently, much reduced in number. The Trigemini, however, as described by Osborn, derives its fibres internally from the following sources: (1) The ascending tract from the cervical region, reinforced by (2) fibres from the

deep motor nucleus, representing two tracts. (3) Fibres from the sensory nucleus. (4) The descending tract from the mesencephalic nucleus. (5) The direct encephalic tract. . . . "The sensory nucleus is very large and extends forwards beyond the level of the cerebellum." It is possible this sensory nucleus is representative, partly, of the lobus trigemini.

In any case, the disappearance of this system of nerves as a separate system in Amphibia in correlation with the disappearance of these cutaneous organs is a most interesting phenomenon. In the Amphibia, then, we have a reduction already accomplished in the disappearance of the end bud nerves, and in the Anura another reduction in process of accomplishment in the loss of the lateral line nerves, in several forms at least, as the fully developed anurous condition is attained.

These questions all have an intimate bearing upon the origin of taste and upon the vexed question of the innervation of the taste buds in the higher vertebrates. It is easily seen that the problem is one of extreme complexity. It is not unreasonable to expect, however, that thorough comparative researches upon the exact composition of the cranial nerves will clear up these obscure points.

Stated briefly, we seem to have the following alternatives: (a) The taste buds (end buds) are innervated in the lower forms by the root from the lobus trigemini only. As the latter diminishes, owing to the loss of the end buds on the exterior, these fibres fuse with the Trigemini proper. This view does not seem to harmonize well with the facts in Amphibia, as stated above, namely, the innervation of end buds by the R. mandibularis internus VII, R. lingualis IX, *etc.* The R. palatinus, and even the R. mandibularis internus VII, might indeed receive the fibres in question through the anastomoses with the V. This would tend to show that the V is the nerve of taste in the higher forms.

(b) The innervation of these structures can be regarded as shifted from one set of nerves to another, *i.e.*, from those issuing from the lobus trigemini as this diminishes or is lost, to those issuing from the lobus vagi (fasciculus communis).

This involves questions hardly settled, as to change of function of nerves, the innervation of the buds being taken up secondarily in this case by sensory visceral nerves.

(c) We may regard, with Mayser, the lobus vagi and trigemini as equivalent structures, in which case there is no difficulty presented by the innervation of the buds by various nerves from these two structures. This ignores, however, the greatly different innervation territory, which the lobus vagi has, in other respects, as compared with the lobus trigemini.

A modification of the latter view seems most probable. The lobus vagi and lobus trigemini cannot be regarded as entirely equivalent structures, but the latter may be considered as devoted to the innervation of the end buds, and the former may be considered to contain similar fibres, in addition, however, to others of a different nature. We have already seen (p. 189) that Mayser distinguishes two sets of fibres, medullated and non-medullated, in the lobus vagi and have also found in the tadpole, in such branches as the R. palatinus VII and R. mandibularis internus VII, two sets of fibres, one somewhat coarser and with a sheath staining much more darkly than the other. This may partly account for the diminishing of the lobus vagi, where the branchial cavities — which are supplied with these buds in fishes — disappear.

One fact is very apparent, both from the investigation of the Amphibia and the comparison with the results of other workers upon the fishes, namely, that there is no direct genetic connection between the lateral line system and its nerves and the sense of taste and its nerve supply. The suggestion of Beard upon this subject has already been criticised in my preliminary communication in the *Anatomischer Anzeiger* (62). Both may have been derived from a more generalized and older form of sense organ, the end bud, present generally on the body and in the mouth. The lateral-line system, whatever its origin, is a specialized system, and its nerves are in every way sharply contrasted with those connected with taste. The auditory organ is the only one whose connection with the lateral-line system is at all probable.

It is also evident that the lateral-line system has no especially segmental character, and that it cannot properly be used in the manner in which it has been attempted to use it, as a general guide in determining the segmentation of the head.

4. *Motor Component.*

The fourth, motor component, shall not be treated here at length. In all the forms above the Cyclostomes we have splanchnic motor roots to the V, VII, IX, and X.

Ahlborn describes no such roots, apparently, except for the V, where it is very large. As Julin (33) and Dohrn (15) assert that the VII has motor elements, it is possible a motor root will yet be found for it. Ransom and Thompson (50) describe large motor fibres, which unite with the Vagus in *Petromyzon*, but not in *Myxine*. These fibres, however, are from anterior roots. There are, besides, exceedingly fine fibres to the heart and blood vessels.

It would appear, both judging from this and from the conditions in the tadpole, that a distinction exists between vaso-motor and branchio-motor nerves, though both are considered visceral.

The following are these motor roots in the authorities mentioned above:

| FORMS. | AUTHORITIES. | NAMES OF ROOTS. |
|---------------------------|----------------------|---|
| <i>Cryptobranchus</i> . . | Osborn | "VII-VIII 3 and 4." |
| <i>Acipenser</i> | Goronowitsch | ventral root of Facialis. |
| <i>Amiurus</i> | Wright | transverse root of VII. |
| <i>Cyprinoids</i> | Mayser | ventral geniculate root of VII. |
| <i>Pleuronectes</i> . . . | Stannius | root 5. |
| <i>Raja</i> | Stannius | part of root 2. |
| Generalized type . | Stannius | root 4. |
| <i>Hexanchus</i> | Gegenbaur | Facialis. |
| <i>Echinorhinus</i> . . | Jackson and Clarke . | "V γ VII." |
| <i>Scyllium</i> | Marshall and Spencer | part of "VII δ "? |
| <i>Laemargus</i> | Ewart | part of hyomandibular and palatine root of VII. |
| <i>Protopterus</i> . . . | Burckhardt | "VIII 3, 4." |

It must be remembered, however, as shown above, that there is reason for believing these roots, except those in *Cryptobranchus* and *Protopterus*, are in reality compound, and include a component from the *fasciculus communis* (*lobus vagi*).

The motor root and the *fasciculus communis* root forms, as we have seen, the constant part of the *Facialis*, including the *portio intermedia*, which persists in the higher vertebrates. It is, of course, this motor root which becomes so much more important in the highest forms owing to the development of the facial musculature.

5. *Comparison with the Cyclostomes.*

It will be well to take a glance at the conditions among Cyclostomes, which, probably, present very primitive conditions, and which should throw much light upon the origin of the various differentiations existing among the cranial nerves of the higher fishes. There have been many contradictory statements made, however, concerning the Cyclostome peripheral nervous system, and I think it will be evident that our knowledge of it is still far from accurate.

Langerhans (40) describes two kinds of cutaneous sensory organs. One is situated in papillae, which are especially numerous around the mouth and on the dorsal "fin," but are also found scattered over the body and in the mouth and pharynx. The other is situated at the bottom of pits, and these pits are arranged irregularly in lines, there being a line above and a line below the gills, other lines proceeding along the side of the body, above the middle; and still other lines around the mouth and over the head, around the eye. These sunken organs he homologizes with the lateral-line system.

According to Ahlborn (1 and 2), the Trigeminal springs from the medulla by three roots, one above the other. The most dorsal is the ophthalmicus, and arises from the ascending trigeminal tract. It has a separate ganglion. The next is the remainder of the sensory Trigeminal, and has the same origin. The most ventral motor root arises from the motor trigeminal nucleus and a descending tract. The ascending Trigeminal is

a direct continuation of the dorsal funiculus of the cord. It consists principally of fine, with a few medium-sized, fibres.

The Vagus roots fall into two divisions. The four roots emerging farthest caudad terminate internally in the "upper lateral ganglion," which is a continuation of the dorsal column of cells in the cord. The four roots emerging most cephalad have the same internal termination as the Acusticus.

The terminal nuclei of the Acustico-facialis, and the four roots just mentioned, lying as they do above the ascending Trigemini and upper lateral ganglion, which represent continuations of the cord, are thus, according to Ahlborn, something superadded in the medulla oblongata, and are equivalent to the higher brain centres. They are not represented by any portion of the cord, or, at the most, by a very small band mesad of the dorsal funiculus, of which band one can only say that it exists. The nucleus of the Facialis lies well above and separated from that of the Acusticus. The cells in it are small and the fibres of the Facialis issuing from it are fine and of a very uniform size. The Acusticus emerges in two roots, one above the other.

Of the IX + X roots, the four composing the first (cephalic) set unite to form R. branchialis I, which sends a twig to the Hypoglossus. The first two of the second set are joined by a recurrent branch from the Facialis to form the fine-fibred N. lateralis. The last two roots form the Pneumogastricus, which is connected with the N. lateralis through the ganglion of the same.

The first fact that impresses one in this arrangement is that the first set of roots from the Acusticus region do not form the N. lateralis, but the R. branchialis I. Furthermore, the N. lateralis is formed partly by a recurrent branch from the Facialis passing around outside the auditory capsule — a thing which does not occur in the N. lateralis in the higher forms. Again, on comparing the course of the N. lateralis with the arrangement of the pits, it is evident that only a small proportion of them would be innervated by this nerve, which has a position near the mid-dorsal line. When these facts are considered — especially the non-derivation of this nerve from the

Acusticus center, thus differing from the origin so universal for the *N. lateralis* in all other forms—it must be regarded as very probable that this nerve does not represent the *N. lateralis vagi* of higher forms. Stannius has also called attention to some of these difficulties and reached a similar conclusion (57, p. 96). What it does represent is probably the *R. lateralis trigemini*, so-called, of Teleosts—a nerve which is formed principally, as we have seen, by a recurrent branch of the *Facialis*, derived from the *lobus trigemini*, and which is reënforced by a branch from the *Vagus*. It would then much more probably innervate the papillae which are so numerous on the dorsal fin, and which probably correspond to the structures innervated by the so-called *R. lateralis trigemini*. The *R. branchialis I* would, apparently, represent the *R. lateralis*. I am forced to believe that the exact anatomy of these nerves is not yet accurately known, nor have their connections with the cutaneous sense-organs been sufficiently worked out. With respect to the remainder of the *Facialis*, it has been asserted by Julin (33) and Dohrn (15) to contain motor elements. If this be true, it probably does not arise merely by the one root, as described.

If the character of the so-called *N. lateralis* be as above supposed, the most dorsal nucleus of the *Acustico-facialis* center, from which the *Facialis* emerges, would correspond to the *lobus trigemini*. Its structure, as described by Ahlborn, would seem to support this view. If this be the case, it is evident that the distinction between the *lobus trigemini*, on the one hand, and the ascending *Trigeminus* and *lobus vagi*, on the other, is already here sharply drawn, and is quite a primitive feature. This would be in opposition to Mayser's views quoted above (p. 189). The course of the *Facialis* in the head is not decisive on this point, inasmuch as we have seen that in higher forms the *lobus trigemini* and lateral-line components go together.

In comparison with Kupffer's observations (36), it would seem probable that, in general, Kupffer's medial elements derived from the neural crest and common to both spinal and cranial nerves, would be represented by such ganglionated nerves as emerge from the ascending *Trigeminus*. It must be remembered, however, that, according to Mayser, not only

the ascending Trigemini, but the lobus vagi and lobus trigemini taken together, represent continuations of the spinal cord; that in the tadpole the lobus vagi (fasciculus communis) can be traced, enormously diminished, into the cord a short distance at least; and that, according to Ahlborn (1), and also according to Ransom and Thompson (50), the Vagus, as well as the Trigemini, in Cyclostomes, come from centers representing continuations of parts, though different parts, of the cord. Mayser (41) and Ahlborn are in accord, however, in considering the Acusticus-lateralis center as something distinct and superadded. Even in Cyclostomes, however, the Vagus center is a considerably enlarged and developed center in the medulla.

Returning to Kupffer, it would next seem probable that his lateral element, which is derived from the dorsal fusion with the epiblast, is represented by the lateral-line element, plus, perhaps, that to the end buds; and that, finally, his epibranchial ganglia are represented by the ganglia of nerves terminating centrally in the lobus vagi (= fasciculus communis + terminal nucleus = fasciculus solitarius + sensory nucleus IX + X). Whether the ganglia of the nerves supplying the end buds can be considered as belonging to or representing this series rather than the preceding is a question whose answer depends upon the answer to the correlated question, already considered, as to the relation between the lobus trigemini and lobus vagi.

IV. GENERAL CONSIDERATIONS.

1. *Relation of Cranial and Spinal Nerves.*

Hatschek (30) has made some interesting and suggestive comparisons between the nerves of *Amphioxus* and *Ammocoetes*, which at the same time throw more light upon the origin of such nerves as the lateralis and visceralis, and of the differences obtaining between the cranial and spinal nerves. In brief, this view is as follows: In *Amphioxus* the dorsal root divides into a dorsal and ventral branch. The dorsal branch subdivides into a N. cutaneus dorsalis and a N. lateralis dorsalis. The ventral branch divides into a N. cutaneus ventralis,

a N. *lateralis ventralis*, and a N. *visceralis*. Only the latter contains motor as well as sensory fibres (motor to the splanchnic muscles). This primitive relation is retained by the cranial nerves, and the loss of this relation and of certain branches by the spinal nerves is owing to the usurpation of the long trunk-branches of the cranial nerves. The course of the spinal dorsal nerves inside the body muscles he regards as due to the shortening of the dorsal nerve. Kupffer, however, regards the spinal dorsal nerve as a new acquisition. This peculiarity presented by the spinal nerves is very important, and its satisfactory explanation must be a crucial point in any theory of the cranial and spinal nerves.

Two general criticisms that may be made upon Hatschek's views, I think, are that they do not take sufficiently into account qualitative differences in the nerves under discussion, his conclusions being based more upon simply topographical relations, and, secondly, that they consider the nerves too much apart from correlated structures, from those innervated by them especially, and, consequently, offer no explanation why the usurpation in question took place.

We have seen that in the cranial nerves of the higher fishes there are three kinds of cutaneous nerves distinguishable by peculiarities of their fibres, of their distribution, and of their internal origin, *i.e.*, (1) mixed fibres of a general cutaneous character continuous with the posterior columns of the cord, (2) coarse fibres innervating the lateral line organs and terminating centrally in the differentiated tuberculum acusticum, and (3) fine fibres innervating the terminal buds (coarse in Selachians and innervating the ampullae?) and terminating centrally (principally) in the lobus trigemini. The latter, *i.e.*, (3), is possibly not completely differentiated. Among the Cyclostomes, it seems probable, this specialization has not been carried so far, but this is not yet sufficiently known.

It is obvious that it is only similar and specialized structures that are most likely to attain a more unified innervation, and, accordingly, it seems most probable that such a process of usurpation as that mentioned above would take place in connection with the cutaneous sense organs. These latter would

concomitantly become more restricted to certain regions and would, not improbably, undergo further specializations. According to this view, the cutaneous sense organs would have had at first a more general innervation, and only later would their nerve supply proceed from only one or two nerves.

Another change which seems to have taken place, similar in character to the above but affecting a different structure, is the assumption by several nerves of the supply to the gills (and other visceral structures?) and concomitantly the creation in the medulla of a special center for this nerve supply (*lobus vagi*).

Hatschek points out the superficial position of the ganglia in *Amphioxus* as compared with the spinal ganglia in vertebrates. If the above homologies (p. 200), with Kupffer's results, be correct, it is precisely the branchio-visceral ganglia of the VII-IX-X (epibranchial ganglia), and the special cutaneous ganglia (lateral line ganglia and ganglia of nerves to end buds also, perhaps) which arise from special epiblastic thickenings as opposed to those ganglia derived from the neural crest, and it is precisely these ganglia which belong to the cranial nerves with long trunk branches, in other words, those which have taken the place of certain portions of the spinal nerves. The ganglia, then, of the cranial nerves, arising in connection with epiblastic thickenings, are the ganglia which the spinal nerves do not possess, having probably lost them. This would explain this difference in the mode of origin of cranial and spinal ganglia.

The fusions described, in connection with the Trigemini, by Beard and Kupffer possibly belong to the lateral line ganglion which lies over the Gasserian ganglion proper, possibly, also, to nerves to end buds.

Of course the presence of the lateral motor roots in cranial nerves constitutes a difference of another character from the above. This and the correlated problem of the sympathetic do not fall within the scope of the present discussion.

There are several anatomical peculiarities which afford further support to Hatschek's comparison, as amended above. These are the remarkable parallelisms existing between these three systems of cutaneous nerves. I have called attention to the

existence of this peculiarity in the relations between certain minor branches of the Trigemini and the lateral line nerves to the head. The parallelism is carried further than this, however. The R. ophthalmicus superficialis VII parallels the R. ophthalmicus V, the R. buccalis VII parallels the R. maxillaris V, the R. communicans IX ad VII parallels the R. mandibularis externus VII, and the postauditory R. supratemporalis parallels the R. auricularis vagi. In fishes this is still more striking where the two pairs first mentioned are in close apposition and can only be separated with difficulty. We have, besides, in fishes the element from the lobus trigemini, whose fibres are not merely in close apposition but, probably, mingle with the lateral-line fibres, forming thereby a still closer union. On the trunk we have the two long branches: the R. lateralis trigemini (facialis) and the N. lateralis.

These parallelisms might be partly explained by the subsection of the nerves in their growth to similar influences by the other parts, but this could hardly explain such close parallelisms. Nor are such parallelisms easily reconcilable with the theory that the lateral-line system of nerves represents a system phylogenetically different from the others. The explanation most naturally suggested is that these three systems were originally one. Of the three, the general cutaneous system to which the Trigemini proper belongs would represent most nearly the primitive nerves from which the others were differentiated. It is, naturally, only the differentiated cutaneous nerves that we find extending over the trunk from the cranial nerves; or, to put it in another way, the spinal general cutaneous nerves bear the relation to the cranial special cutaneous branches to the trunk that the general cutaneous branches from the ascending Trigemini bear to such branches in the head. We might even advance a step further and say that the more intimate fusion of the end bud and lateral line nerves indicates the evolution of the latter from the former, a view probable on other grounds and advocated in Wiedersheim's *Grundriss* (67).

Hatschek (30) has called attention to the morphological importance of the dorsal rami as landmarks of former conditions.

The other longer and principal branches have been more influenced and extended by the development and topographical changes in various parts, such as the jaws, sense organs, *etc.* Hatschek, however, as we have seen, does not recognize the fact that there are, even of these dorsal rami, qualitatively three kinds. In determining segmentation, by means of them, it is necessary to decide first, whether each can represent a separate segment, or all must be taken together to represent one segment. The former view would rest upon the supposition that some of the components for any one segmental ramus have been lost. The latter view would, perhaps, seem more probable, and agree better with the views here advanced as to the cause of these differentiations of cutaneous nerves.

It is questionable whether these components belong especially to different levels of the head and body, as Hatschek's comparison with *Amphioxus* would seem to indicate. Each one of his four sensory branches might consist, actually or potentially, of the three components. From the actual relations in fishes, however, the general cutaneous component is naturally confined to no particular locality in its innervation territory. Its roots are ventral to the other two. Of the other two, in origin, the lateral line component is ventral to the remaining one, and is lateral in its general distribution on the body. On the head there is no difference, topographically, in this particular respect, between the three cutaneous components.

If Hatschek's view, similar in some respects to Balfour's, is correct, and the cranial nerves most closely approach the primitive conditions, the spinal nerves having lost elements, then we would naturally find these tracts, peculiar to the medulla, showing the remnants, at least, of continuations in the cord. Whether this were true or not, however, the validity of this view would hardly be affected.

It is useless to speculate further upon this subject. What it is wished, however, to emphasize here, is the importance of taking into full consideration, as a factor, the cutaneous sense organs, in the attempt to obtain a philosophical understanding of the changes undergone by the peripheral and central

nervous systems. The development and specialization of these structures have probably played an important part in the changes leading to the organization of the vertebrate peripheral and central nervous systems. Furthermore, when we come to compare the nervous systems of fishes and Urodela with the higher vertebrates, without the general clue that these structures have disappeared, and the nerves supplying them likewise either disappeared or metamorphosed, only false conclusions are inevitable.

Nor is the question one merely affecting the peripheral nervous system, inasmuch as it affects likewise the central terminations of this nerve supply and indirectly other portions of the central nervous system in physiological connection therewith. A number of changes in the higher centers will probably be found to be connected with these transformations.

As an instance of the effect of these changes upon the central nervous system, aside from the medulla, it is possible, I think, that the reduction of the cerebellum in Amphibia may be correlated, to some extent, with the reduction in these cutaneous organs. Its size in the higher forms would be, perhaps, secondarily reacquired.

As these changes in cutaneous organs are largely affected by habitat, it is obvious that animals widely separated may, by changing their habitat, undergo changes in their nervous system quite similar in character. Here, again, we meet the phenomenon of parallel changes in different forms, due to similar conditions of environment, and, in such cases, of physiological rather than morphological value.

2. *Relations of the Pre- and Postauditory Nerves.*

A peculiarity to which attention may be called here is the position of the Auditory among the nerves.

It is evident, from the description of the nerves of the tadpole, that the pre- and postauditory nerves are not totally different by any means; yet, in general, there are marked differences in the relative development of the different components corresponding to the differences in the character of

the innervation regions. In the preauditory nerves we have a large general cutaneous element for the supply of the extensive surface of that portion of the head, while the preauditory supply for visceral surfaces is naturally comparatively small. We have a large visceromotor supply, however, for the enlarged branchial (jaw) musculature. In the postauditory region the general cutaneous supply is small, owing to a reduction of the surface supplied, due to the breaking through of the gills, and also possibly to encroachments by trunk nerves. On the other hand, the visceral surfaces supplied are extensive and these nerves correspondingly developed.

The manner in which the auditory organ is interposed, as it were, is evidenced by the many anastomoses around it. In the tadpole, while one half of the lateral-line nerves is related to the V, the other half comes into relation with a general cutaneous nerve from the IX + X (R. com. IX + X ad VII), which unites with it in a manner similar to that in which the first half of the lateral nerves unite with the V, and between their respective branches similar parallelisms seem to exist.

While the trunk nerve to the lateral line issues with the IX + X, the parallel trunk nerve to the end buds of the trunk (R. lateralis trigemini) has a preauditory exit. Furthermore, among the fishes, the palatine nerve would appear to be formed by a union of post- and preauditory nerves (Goronowitsch, Pollard). The development of the auditory organ has probably caused a separation of nerves formerly more closely connected. This is evidenced also by the manner in which the lateral-line nerves converge mesad of the auditory capsule just before entering the medulla. Ayers (6) has brought forward reasons for supposing that the acustico-lateralis system was originally double. We might even go a step further and suggest the possibility that it was once single.

In any case, the general relations of the pre- and postauditory nerves point, I think, to the conclusion that the auditory organ is a neomorph interposed among the nerves and altering their primitive courses.

3. *Bearings upon the Classification and Segmentation of the Nerves.*

The foregoing comparative study of the cranial nerves shows that *the present numerical classification is unphilosophical.*

One principal cause lies in the fact that the classification, with its serial numbering of the nerves, is based upon the conditions existing among the higher vertebrates. Now, as we have seen, the cranial nerves of the higher vertebrates have undergone considerable reduction of primary components.

Much time has been spent in ascertaining whether those preauditory roots, issuing from the tuberculum acusticum and lobus trigemini, belong to the V or VII nerves. For convenience and to emphasize their distinction from the V they have been considered in this paper as belonging to the VII, in accordance with recent researches. If, however, we take the cranial nerves of the higher vertebrata as a basis, which is practically done in the existing nomenclature, these components or roots in question do not belong to either the Trigemini or Facialis; they are actually different nerves from either of the above, existing in the lower but not in the higher vertebrates. The principal reason they have been assigned by recent investigators to the VII is because their branches have been shown to originate from these roots lying caudad of the Trigemini proper, and, consequently, by implication, belonging to the segment of the VII. This, however, is not logical, inasmuch as segmental character is not the basis of existing nomenclature, nor, indeed, would it be possible with our present knowledge to propose a nomenclature for the cranial nerves on this basis. Furthermore, it has not actually been determined to what segment or segments these special cutaneous roots belong.

It will therefore ultimately be necessary to remodel our cranial nerve terminology, but, in my opinion, their exact composition has not yet been sufficiently determined in order to do this successfully.

It may be well to indicate here the weak point in Gaskell's analysis of the nerves (23 and 24), namely, that it does not take account of all the qualitative differences among them.

For example, the lateral-line system is obviously different, whatever may have been its origin, from such cutaneous nerves as the Trigemini, yet in Gaskell's nomenclature they are both simply classed together as ganglionated afferent somatic nerves. In fact, Gaskell's system also ignores the differences between these nerves and those of the special sense organs, for these latter would also fall under the above category. The chief defects in Gaskell's work appear to arise from the fact that it has been confined to the highest vertebrate types, that it is not comparative. Nevertheless, it is upon lines of work approaching those of Gaskell that, in my opinion, the most fruitful results will be obtained.

It is also evident that an exact determination of the component parts of the nerves is a necessary preliminary step to dealing with questions of segmentation. This fact has already been recognized to a certain extent, as is evidenced by the numerous attempts to find the corresponding ventral roots for dorsal roots, and *vice versa*. In these attempts, however, many other differences in the character of the nerves have been ignored. His, Van Wijhe, Gaskell, and others have demonstrated the presence of two sets of motor nerves, and it is evident that further differences, especially in the sensory nerves, must also be taken into account. For example, in the tadpole, the Trigemini proper, the "dorsal VII" and the "fasciculus communis" root of the VII, are mainly sensory yet all different; either all three must be included in a complete "segmental nerve" or, if one is omitted, it must be shown why. That some particular component may disappear in certain cases is evident, and the cause is then to be sought in some peripheral change.

In the study of segmentation from the standpoint of the neuromeres, as developed by Béraneck, Orr, McClure, Waters, Platt, and others, these qualitative differences should likewise be taken into account. When the results of these two lines of work shall have been brought into correlation with each other, a better insight will be afforded, on the one hand, into the significance and value of the neuromeres, and, on the other hand, into the transpositions and other changes undergone by the various components.

Finally, without a recognition of these nerve components, embryological research, it appears to me, becomes partly meaningless. The fact that certain ganglia are connected, in course of development, with epiblastic thickenings while others, apparently, are not, is correlated with some difference, structural and physiological, which either exists now or has existed. How can we determine what value to attach to such differences of origin until we know with what differences in the adult structure they are correlated and, as a consequence, what was the original cause of their appearance? A knowledge of the structure itself may be fairly considered a necessary preliminary to ascertaining its embryological origin.

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APPENDIX ON TECHNIQUE.

Although nearly every one who takes up any especial line of work evolves, to a certain extent, his own technique, and although the Golgi method is described in a number of articles and books, yet it may be well to give the details of manipulation, as found convenient in my experience, to benefit those who are not familiar with the subject and literature.

1. *Hardening of Small Pieces in Osmium-bichromate.*—The size of the pieces will naturally depend upon the character of the tissue, but, as a rule, one dimension should not exceed 3 to 4 mm. Perhaps the best mixture for general use is potassium bichromate, $3\frac{1}{2}\%$, 4 vols. + osmic acid, 1%, 1 vol. The time of hardening must be largely a matter of experience, depending upon such factors as the character of the tissue, its stage of development, whether embryonic or adult, temperature, and the character of the impregnation aimed at. In general, this time will lie between 2 and 5 days, embryonic tissue requiring less than adult. For the cortex of an 8-months' human embryo, however, I have found from 3 to 5 days—and especially the latter period—gives the best results when using Berkley's mixture. 30 to 50 cc. are required for a medium-sized tadpole. It is best to put the specimen in a smaller quantity first,—a solution that has been used once will answer,—and then change after an hour or so, putting the specimen in the full quantity. The fluid should be changed at any time if it becomes cloudy or ceases to smell quite strongly of the osmic acid.

2. *The Silver Bath.*—1% may be taken as a standard solution. The pieces of tissue should be washed in several changes of the silver solution (that which has been used once being available), until, after 10 to 15 minutes, the fluid ceases to cloud up with the silver chromate, which is formed when the bichromate and silver solution meet each other. The pieces should be left in a liberal supply of the silver solution—at least double the quantity of osmium bichromate which has been used. Impregnation takes place in 24 to 12 hours, or even less, but it is well to allow the tissue to remain in the silver several days in the dark. Keeping the tissue in the dark in 1. and 2. is not really essential to obtain the reaction, but is preferable, especially if it remains in the silver bath some time (see below).

3. *Cutting and Mounting.*—(a) The pieces are transferred immediately, and left $\frac{1}{2}$ to 1 hour in 95% alcohol, this being changed several times in the meanwhile; (b) next $\frac{1}{2}$ to 1 hour in absolute alcohol; (c) 10 to 15 minutes in alcohol and ether, equal volumes; (d) $\frac{1}{2}$ to 1 hour in thin celloidin; (e) a few minutes in thick celloidin; (f) mounted on a microtome block, and the celloidin hardened in chloroform. This process applies especially to the tadpole, which, when put in the alcohol, is cut transversely into several more pieces to facilitate the washing and, especially, the penetration of the celloidin. For solid tissues, especially the central nervous system, it is better simply to gum them to the block after the washing in alcohol, using celloidin and hardening it by only a short immersion in

chloroform or 80% alcohol. A partial infiltration with celloidin and hardening the latter is apt to crack the tissue inside. With the tadpole and similar tissues it is necessary to fill up the interstices with celloidin in order to obtain complete and coherent sections. When this is done, chloroform is, in my opinion, the best fluid to harden the celloidin. It does this quite rapidly, and does not, for some time at least, seem to affect the impregnation. Specimens may be often left in it over night, apparently without injury.

The sections, 50 to 70 μ thick, usually, are removed from the knife with a camel's-hair brush or piece of tissue paper, and arranged on the slide. 95% alcohol is used in the cutting. The alcohol is then blotted off by gently pressing a piece of filter paper on the slide, and a few drops of absolute alcohol are put on. This is then carefully drained off,—not blotted as before,—and all superfluous alcohol allowed to evaporate, though the sections should not be allowed to dry. The celloidin, softened by the absolute alcohol, will then adhere to the slide during the remaining treatment. The sections are then cleared by means of *ol. origanum cretici*, and the latter is washed off with xylol. They are then mounted in dammar balsam, without a cover slip. The dammar is used in the condition in which it is obtained—a thick fluid. After it is spread over the sections it must be watched for a while, as it tends to run off the sections and accumulate around the edges of the mount, probably owing to diffusion currents. The aim should be, in covering with balsam, to get an even layer of balsam, *as thin as is consistent with covering the sections*. If too thick it does not dry rapidly, even in the oven, and where yellowing takes place subsequently it is most liable to occur where the balsam is thickest. When mounted, the slides are put in an oven, at about 50° C., for a day or two, care being taken that they shall be level. Heat does not seem to affect the preparations, and the sooner the balsam is dried the better. If the balsam should dry off the sections in spots leaving them exposed, the dry places should be first moistened with xylol and then balsam added as required, and the slide dried again in the oven. In my experience, *origanum* and xylol are much preferable to creosote and turpentine, which are recommended by Golgi. This is especially true with the central nervous system, where turpentine tends to crack the sections. The *origanum* does not allow the balsam to dry so readily as does xylol and will also, after a while, affect the impregnation, hence the washing with xylol.

The best way to mount loose sections is to transfer them from the xylol into a dish of quite thick balsam instead of immediately to the slide. In this way the above-mentioned diffusion currents upon the slide are avoided.

Note.—Since the above writing, I have found that the period of hardening may be reduced to a day or so and yet good impregnations of adult brains be obtained also by adding *formalin* (e.g., 4%) to a potassium bichromate solution. How this will compare with the lithium bichromate method, I can hardly, as yet, say.

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ABBREVIATIONS USED IN THE PLATES.

| | | | |
|---------------------------|---|---------------------------|--|
| <i>Anast.</i> | anastomosis. | <i>R.</i> | ramus. |
| <i>aud. cap.</i> | auditory capsule. | <i>Rr.</i> | rami. |
| <i>aur.</i> | auricle. | <i>r. com. IX ad VII.</i> | ramus communicans glossopharyngei ad facialem. |
| <i>Bid. gang.</i> | Bidder's ganglion. | <i>r. lat.</i> | ramus lateralis. |
| <i>bl. ves.</i> | blood vessel. | <i>r. mand. int. VII.</i> | ramus mandibularis internus facialis. |
| <i>cap.</i> | capillaries. | <i>r. pal. VII.</i> | ramus palatinus faci- alis. |
| <i>cart.</i> | cartilage. | <i>r. visc. X.</i> | ramus visceralis vagi. |
| <i>ch. tymp.</i> | chorda tympani. | <i>silv. prec.</i> | silver precipitate. |
| <i>con. tis.</i> | connective tissue. | <i>subepith. n. plex.</i> | subepithelial nerve plexus. |
| <i>cup.</i> | cupula. | <i>sup. gl. n. plex.</i> | superficial glandular nerve plexus. |
| <i>cut.</i> | cutis. | <i>symp.</i> | sympathetic. |
| <i>cut.¹</i> | outer layer of cutis. | <i>term. n. plex.</i> | terminal nerveplexus. |
| <i>cut.²</i> | middle layer of cutis. | <i>vent.</i> | ventricle. |
| <i>cut.³</i> | inner layer of cutis. | <i>Z.</i> | in Pl. XII, key, is placed on points of fusion between ac- cessory branches of the Trigeminus and lateral-line nerves. |
| <i>dors. VII.</i> | dorsal VII. | <i>2 root IX + X.</i> | Second root of Glos- sopharyngeus and Vagus. |
| <i>epid.</i> | epidermis. | <i>3 root IX + X.</i> | Third root of Glos- sopharyngeus and Vagus. |
| <i>epith.</i> | epithelium. | <i>4 root IX + X.</i> | Fourth root of Glos- sopharyngeus and Vagus. |
| <i>epith. plex.</i> | epithelial nerve plexus. | <i>V asc.</i> | Ascending tract of the Trigeminus. |
| <i>fasc. com.</i> | fasciculus communis. | | |
| <i>fib. Mauth.</i> | fibre of Mauthner. | | |
| <i>gang.</i> | ganglion. | | |
| <i>gang. Gass.</i> | ganglion Gasseri. | | |
| <i>gl. n. plex.</i> | glandular nerve plexus. | | |
| <i>inf. gl. n. plex.</i> | inferior glandular nerve plexus. | | |
| <i>intermed. n. plex.</i> | intermediate nerve plexus. | | |
| <i>mot.</i> | motor. | | |
| <i>musc.</i> | muscle. | | |
| <i>oes.</i> | oesophagus. | | |
| <i>perich. plex.</i> | perichondral nerve plexus. | | |
| <i>pigm. c.</i> | pigment cell. | | |
| <i>post. long. fasc.</i> | posterior longitudi- nal fasciculus. | | |
| <i>prot. proc.</i> | protoplasmic process. | | |

EXPLANATION OF PLATE VII.

FIG. 1. Vertical section through the epidermis of a tadpole, showing terminations of the Trigemini. $\times 155$.

FIG. 2. Somewhat oblique vertical section through the oral epithelium, showing the terminal plexus of the R. maxillaris V (+ palatinus VII?). $\times 192$.

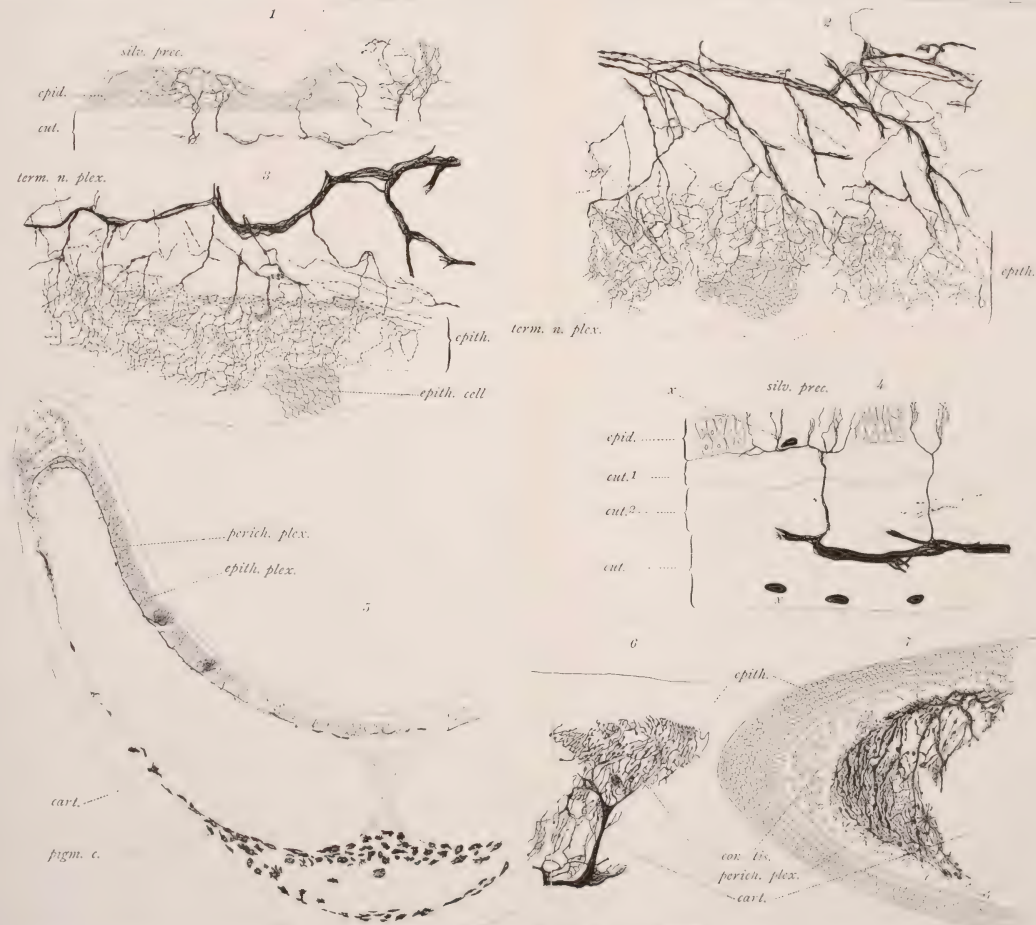
FIG. 3. Section similar to Fig. 2, showing the terminal plexus of the R. ophthalmicus V (+ palatinus VII). $\times 192$.

FIG. 4. Section similar to Fig. 1. $\times 192$.

FIG. 5. Section through the lower labial cartilage, showing the perichondral plexus and the intraepithelial plexus arising therefrom of the R. mandibularis V. $\times 72$.

FIG. 6. Horizontal section through the above-mentioned perichondral nerve plexus.

FIG. 7. Similar section through the above, showing also the entire end of the labial cartilage. $\times 315$.



EXPLANATION OF PLATE VIII.

FIG. 8. Vertical transverse section through a part of the roof of the pharynx, passing through the transverse fold of the epithelium and showing the terminations of a branch of the R. palatinus VII in the epithelium and taste bulbs (end buds). $\times 58$.

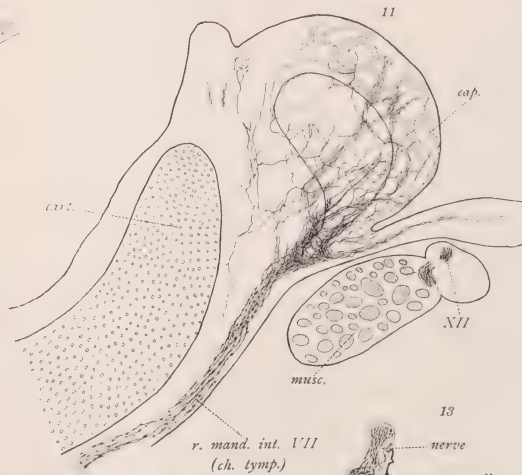
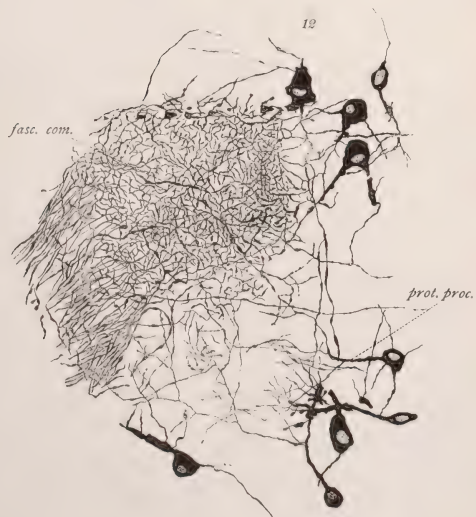
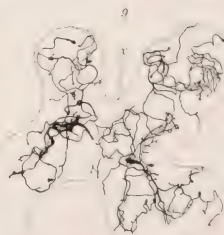
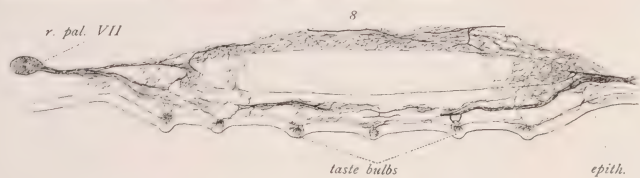
FIG. 9. Terminations of one of the Rr. branchiales X in the terminal pockets of the filtering apparatus. $\times 424$.

FIG. 10. The next section caudad of the one drawn in Fig. 8. $\times 58$.

FIG. 11. Transverse section through the pharynx, showing the terminal ramifications of the R. mandibularis internus VII (chorda tympani) in a large papilla in the lateral angle of the pharynx. $\times 41$.

FIG. 12. Transverse section through the fasciculus communis. $\times 315$.

FIG. 13. Section showing innervation of a small blood vessel by fibres from one of the ganglion cells found along the course of the visceral nerves. $\times 111$.



EXPLANATION OF PLATE IX.

FIG. 14. Longitudinal (frontal) section through the heart, showing the ramifications of the Rr. cardiaci X.

FIG. 15. Vertical section through the pharyngeal epithelium, showing the innervation of a taste bulb (end bud). $\times 315$.

FIG. 16. Similar to Fig. 15.

FIG. 17. Vertical section through the epithelium of the roof of the pharynx, showing the innervation of the multicellular glands of F. E. Schulze. $\times 155$.

FIG. 18. Section similar to Fig. 17, showing the superficial glandular nerve plexus. $\times 265$.

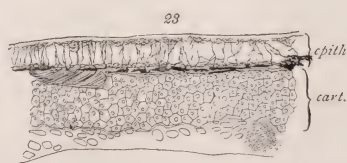
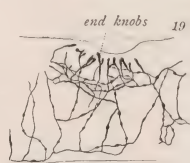
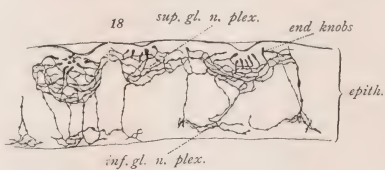
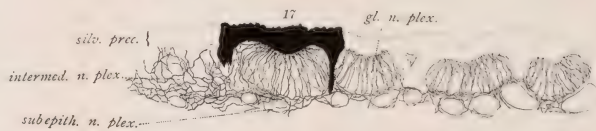
FIG. 19. Similar to Fig. 18. $\times 315$.

FIGS. 20 and 21. Similar to the preceding and showing more precisely the individual nerve endings. $\times 424$.

FIG. 22. Horizontal section through superficial glandular nerve plexus. $\times 192$.

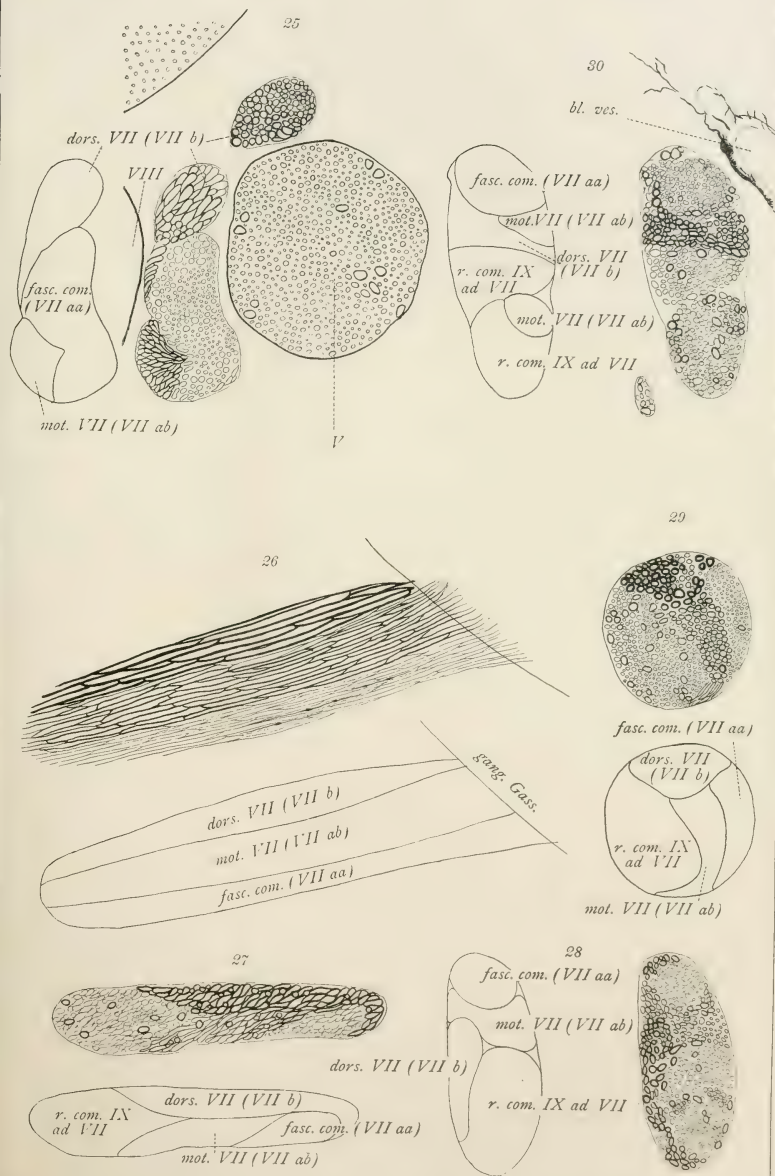
FIG. 23. Vertical section through the epithelium of the roof of the pharynx, showing the continuous superficial glandular nerve plexus where the glands are continuous. $\times 72$.

FIG. 24. Horizontal section through pharyngeal epithelium passing through the cupula of a taste bulb. $\times 315$.



EXPLANATION OF PLATE X.

FIGS. 25-30. Sections through the Hyomandibularis VII at different points along its course, proceeding cephalad (distad), according to the numbering, and showing the components of the nerve. The outlines accompanying each section are to show more clearly the position of the components and to facilitate their designation. Fig. 26 is an oblique section; the others are transverse. $\times 137$.



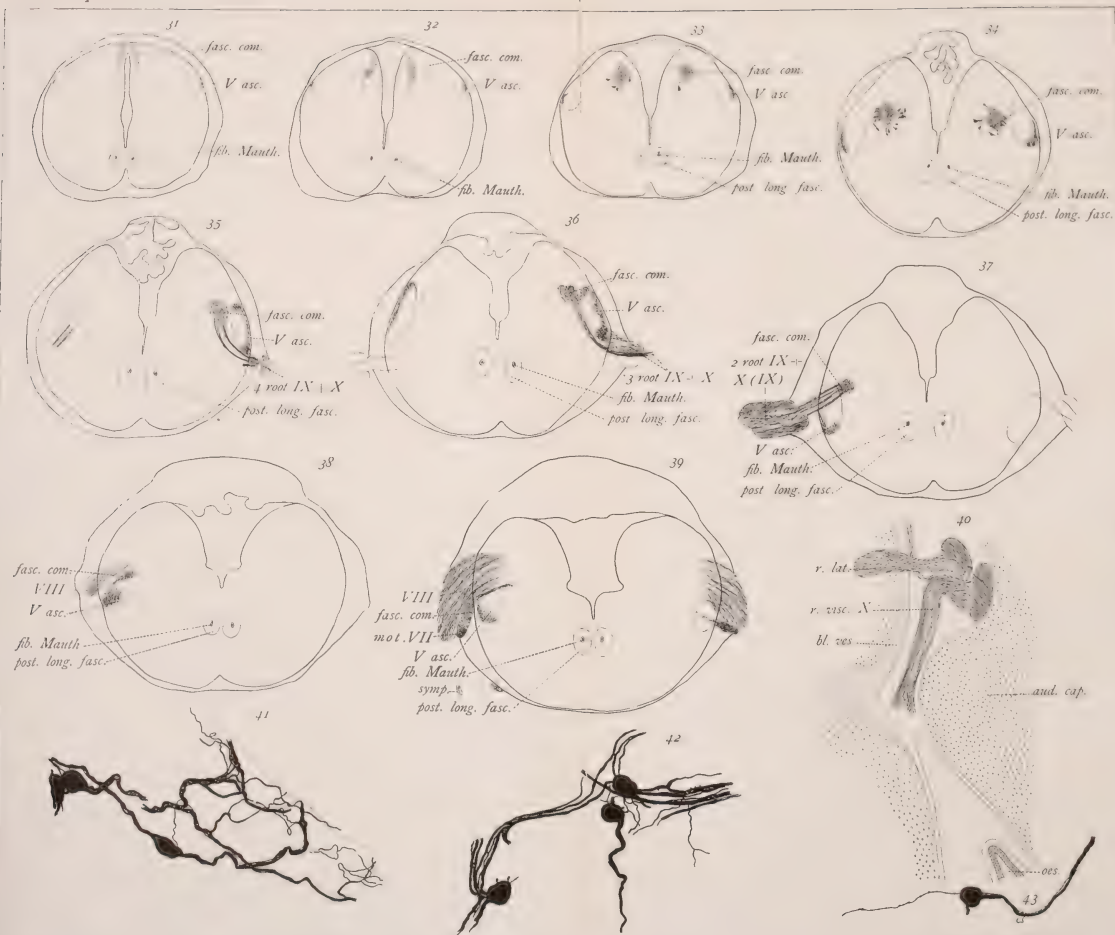
EXPLANATION OF PLATE XI.

FIGS. 31-39. Transverse sections through the medulla proceeding cephalad. They show the position of the fasciculus communis, ascending V and, incidentally, the posterior longitudinal fasciculus and fibres of Mauthner.

FIG. 40. Transverse section through that portion of the vago-glossopharyngeal complex lying just outside the auditory capsule.

FIGS. 41-43. Some of the nerve cells found along the course of the ramifications of the visceral nerves beneath the epithelium of the pharynx. The dotted line in Fig. 41 indicates the inner boundary of the epithelium.

FIGS. 1, 2, 4, and 17 are from preparations by the ordinary rapid Golgi method, somewhat modified, in some cases, in the proportions in the fluids used. Figs. 5, 6, 7, 18, 19, and 24 are from a series prepared by the triple impregnation modification of Cajal. Figs. 3, 8, 10-16, 31-39, and 40 are from a series prepared by means of the sulphate modification. Figs. 9, 20-23, and 41-43 are from other series prepared by the same modification. Figs. 25-30 are from preparations in which the nerves are stained with osmic acid.



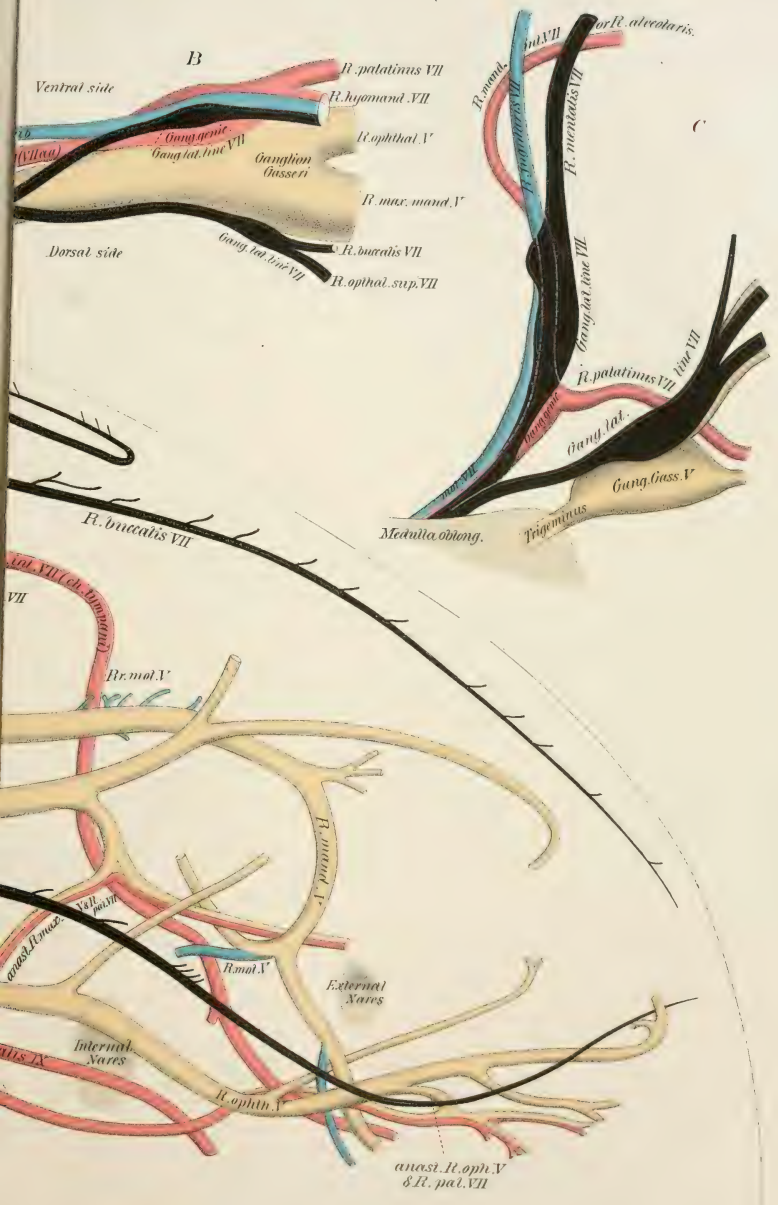
EXPLANATION OF PLATE XII.

A. A reconstruction of the V, VII, IX, and X nerves of the tadpole, showing the different components of these nerves in different colors. One half the head is shown in a horizontal plane and dorsal aspect (see also text, p. 107). $\times 35$.

B. A reconstruction showing the roots of the V, VII, and VIII nerves, the first two as far as the Gasserian ganglion. Lateral aspect.

C. A reconstruction showing the roots of the V and VII nerves in the *Amblystoma* larva. Dorsal aspect.

The eye, ear, and brain are indicated in faint neutral tint, with dotted outlines, portions of the brain being omitted, however, where it overlaps certain of the nerves.



THE CENTRAL NERVOUS SYSTEM OF DESMOGNATHUS FUSCA.

PIERRE A. FISH.

CONTENTS.

INTRODUCTION.

| | PAGE |
|--|------|
| ACKNOWLEDGMENTS..... | 249 |
| DESCRIPTION OF DESMOGNATHUS FUSCA..... | 250 |
| TECHNIQUE..... | 252 |

MORPHOLOGY.

| | |
|---------------------------|-----|
| SURFACE ANATOMY..... | 253 |
| MENINGES..... | 255 |
| BLOOD SUPPLY..... | 257 |
| CRANIAL NERVES..... | 259 |
| GENERAL MORPHOLOGY..... | 261 |
| <i>Rhinencephal</i> | 263 |
| <i>Prosencephal</i> | 264 |
| <i>Paraphysis</i> | 270 |
| <i>Diencephal</i> | 271 |
| <i>Mesencephal</i> | 272 |
| <i>Epencephal</i> | 272 |
| <i>Metencephal</i> | 273 |
| <i>Myel</i> | 273 |

HISTOLOGY.

| | |
|------------------------|-----|
| GENERAL HISTOLOGY..... | 275 |
| FIBER TRACTS..... | 286 |
| SUMMARY..... | 291 |
| BIBLIOGRAPHY..... | 293 |

ACKNOWLEDGMENTS.

It is a pleasure to acknowledge the friendly interest and kind courtesy shown to me by the staff of the Anatomical Department (Cornell University) throughout the prosecution of my research. For the aid that I have received it is quite

impossible to refer in all cases to specific publications ; some of the most valuable assistance has come from conversations and from the many instructive lectures delivered by Professor B. G. Wilder in general neurology. I am also under considerable obligation to Professor S. H. Gage for coöperation and information concerning the salamander that has been studied ; and to Mrs. Gage for the use, by way of comparison, of her unusually instructive series of sections through the head of *Diemyctylus*, as well as for many useful suggestions in connection with my drawings. The abundance and availability of the well selected neurological literature in the University library have also greatly facilitated my work.

DESCRIPTION OF DESMOGNATHUS FUSCA.

So far as the writer knows nothing has ever been done upon the central nervous system of *Desmognathus fusca*, although it is described by Cope as being "perhaps the most abundant salamander in North America." It ranges throughout the eastern district of the nearctic region and, according to Heilprin, is entirely restricted to the western hemisphere. It is commonly known as the "dusky salamander" on account of its dark color which ranges from a dark brown to black on the back and is light or marbled on the belly. It has prominent eyes and a somewhat compressed tail.

This form was chosen, not merely on account of its easy accessibility, but because its habits and activities in many ways represent conditions intermediate between terrestrial and aquatic urodeles ; and it may therefore be considered as representing a fairly accurate type for this group.

The adaptability of this animal to either aquatic or terrestrial environment was tested in the following way: a newly caught and vigorous specimen was immersed in a glass jar filled with water from the tap ; in the mouth of the jar was placed a strainer with its convex bottom projecting under the surface of the water, thus preventing the salamander from getting any air except that mixed with the water. He was kept in this way and without food for two weeks, the water being changed

each morning and night. He was then "marooned" for a week or so in a jar containing nothing but some damp earth and food. After his restoration to his mates in the aquarium he appeared as active as any of them.

These experiments were carried on early in 1892 and they are of peculiar interest from the fact that in the many specimens dissected no trace of lungs were ever seen, nor are gills present except in the larval stage. The epithelium of the mouth cavity, except the tongue, is of a ciliated columnar character; the œsophagus is likewise ciliated, the movement being in a caudal direction. Gage (Proceedings of the American Association for the Advancement of Science, Vol. XXXIX, 1890, p. 338) finds that "In all forms of Amphibia and in all stages after the complete disappearance of the food yolk, ciliated epithelium is absent from the mouth when the respiration is mostly aquatic, and water is frequently taken into the mouth; and that in forms with mostly aerial respiration, where water is rarely taken into the mouth, the mouth is lined with a ciliated epithelium."

In the aerial respiration of the *Desmognathus* the floor of the mouth is alternately raised and lowered very rapidly, while during his enforced aquatic sojourn it was noted that he raised the floor of his mouth and kept it so for a long time. It is not impossible that in this form aquatic respiration may predominate from late fall to early spring, and aerial during the summer months. If this be the case we might expect the ciliated or non-ciliated condition in accordance with what has been said above.¹

Although the neurology of the urodeles has been enriched by valuable contributions from eminent investigators both in this country and in Europe there has been scarcely any appli-

¹ In the *Anatomischer Anzeiger* for January, 1894, No. 7, pp. 216-220, under the title of Lungenlose Salamandriden, Dr. Harris H. Wilder states that the *Desmognathus fusca* may live some forty-eight hours away from the water, in a box filled with fresh grass. He does not comment upon the oral or œsophageal epithelium, but his figures show it as columnar and non-ciliated. My own experiments show that these salamanders will live for weeks in a small jar, with no other water than that present in a small piece of moist sod left in the jar with them; a small amount of water being added from time to time to prevent its drying up.

cation to these forms of that most useful of modern neurological methods: the Golgi-Cajal short silver nitrate method. Since this has in so many ways revolutionized our ideas of the structure of the nervous system of higher animals it seemed to me more than likely that we should find in these more generalized types conditions that ordinary methods have hitherto failed to demonstrate.

TECHNIQUE.

For the morphological study, sections in most cases were made through the entire cranium or head, after it had been decalcified. Perenyi's mixture or Gage's decalcifier was used for this purpose. The latter is made up by adding 3 cc. of strong nitric acid to 100 cc. of 70% alcohol, and gave uniformly good results.

The tissue was fixed in various ways: Potassium bichromate, corrosive sublimate, picric alcohol, and a mixture which may be called picro-aceto-sublimate composed as follows: 50% alcohol 1000 cc., glacial acetic acid 5 cc., corrosive sublimate 5 grams, picric acid 1 gram. This gave the most satisfactory results and brought out many histological details that were not demonstrable by the other methods. Gage's picric alcohol is the basis of the mixture, the other parts being added to give a more precise definition to the cells and their parts. For delicate objects it is well to dilute the above with an equal volume of water.

The tissue according to its bulk is well fixed in from twelve to twenty-four hours, and the sublimate and picric acid are washed out in 50% and 70% alcohol. For staining, Delafield's hematoxylin gave most excellent results; contrast staining with Van Gieson's picro-fuchsin gave most brilliant effects. Herrick's modification was found to work very satisfactorily. It consists of adding an antiseptic tablet (corrosive sublimate .5 gram and ammonium chloride .5 gram), to about half a liter of the stain. Another satisfactory modification is the addition of 1 cc. of glacial acetic acid and 1 cc. of a saturated aqueous solution of corrosive sublimate to 100 cc. of the hematoxylin.

For histological detail, the short silver nitrate method of Golgi, and Weigert's hematoxylin method were mainly employed. In the silver method the following proportions were used: 3% potassium bichromate 100 cc., 1% osmic acid 20 cc. The tissue was allowed to remain in this mixture from twenty-four to forty-eight hours, according to the temperature; it was then rinsed rapidly and placed in a weak solution ($\frac{1}{4}$ %) of silver nitrate for fifteen or twenty minutes. This was changed a couple of times until the fluid remained clear. It was then immersed in a $\frac{3}{4}$ % silver nitrate solution, and left there for two days or longer. The addition of a drop of formic acid to every 100 cc. of the silver solution, in order to facilitate reduction, as recommended by Van Gehuchten, was tried, but with no apparent advantage or disadvantage. Single and double impregnations of the silver were tried and very good effects were obtained from each. It seemed as if the latter gave more constant results. Collodion imbedding was employed throughout. The object was cleared and cut in the castor-thyme oil mixture: red oil of thyme, 3 parts, castor oil 1 part.¹

If the specimen is stained *in toto*, the method is a very expeditious one; and it was found that the sections, after absorbing the superfluous oil with tissue paper, could be fastened to the slide by means of a drop or two of ether alcohol, and that they might then be passed through the various alcohols and stained similarly to the paraffin or ordinary collodion methods. Any tendency toward crumbling or tearing on the part of the sections may be obviated by painting the cut surface of the object with a thin layer of one per cent collodion before making each cut. This will also enable one to cut much thinner sections.


SURFACE ANATOMY.

Macroscopic methods are of but little use, the average size of the brain being about one and one half to two millimeters in width and six to eight millimeters in length; this in con-

¹ P. A. FISH. A New Clearer for Collodionized Objects. *Proceedings of the American Microscopical Society*. XV, pp. 86-89. 1893.

nection with the difficulty of removing so small an object without injury, renders microscopical methods a necessity. The study of the general topography, the relations of the cavities and other parts is greatly facilitated by the use of a series of enlarged camera lucida drawings or the construction of models in wax or otherwise.

Barring its size, the general simplicity of the amphibian brain would render it a most admirable object for the study of morphological relations; its general absence of flexure, its successive segmental arrangement and the degree of exposure and differentiation of these segments, give it a great advantage over most other generalized forms. The brain of *Desmognathus fusca* is a slender elongated organ, its widest point being at the latero-caudal ends of its hemispheres or at about the middle of its length; the general dorsal surface is nearly on the same plane, most of the segments being exposed. The lateral regions, especially of the prosencephal, slope quite abruptly toward the ventrimeson (ventral median line), so that if a transection be made in this region a triangular outline will be the result, the base being dorsal and the apex ventral. The ventral aspect is much more broken and irregular.

There is no evidence of a cranial flexure; the pons flexure persists and there is likewise some evidence of a neck or cervical flexure. Assuming that the diencephal and mesencephal are the fixed portions of the brain axis, and that there is a greater rate of growth of the parts cephalad and caudad of them, we can perhaps arrive at some explanation of the arrangement of the parts. The metencephal in its more rapid growth longitudinally would tend to grow under and around the caudal half of the mesencephal and become overlapped by it; while in the case of the prosencephal, during its dorso-caudal growth the hemispheres would tend to divaricate and overlap the diencephal to some extent laterally and give the latter a wedge-shaped appearance. The whole brain then, as seen from the side, might compare slightly in outline with a . So that in this form there is a departure from the straight and unflexed condition as exemplified by the most of the aquatic urodeles. On the dorsal aspect, the large supra-

plexus lies between the hemicerebrums at about the point where they begin to diverge. Just caudal to the plexus is a slight circular mesal elevation, the epiphysis. This, in some specimens fixed in potassium bichromate, presented a white or silvery appearance due to the presence of a pigment.

There is no sharp line of demarcation between the diencephal and mesencephal; the latter is approximately circular in outline and in some specimens a slight mesal furrow has been detected; this, however, is believed to be an artifact due to the collapse of the roof rather than to represent the normal convex condition usually observed. The cerebellum is not visible, being entirely overlapped by the mesencephal. The metaplexus is comparatively short and wide as compared with other *Amphibia*.

On the ventral aspect, the olfactory nerves pass off from the latero-cephalic portion of the cerebrum. There is no very marked indication of an olfactory lobe superficially; a very slight furrow more noticeable on the ventral aspect passing obliquely latero-caudad, seems to differentiate these lobes from the cerebrum proper. A small whitish area between the intercerebral fissure and the chiasma indicates the position of certain of the cerebral commissures. The chiasma is not prominent; it scarcely projects beyond the ectal surface, and topographically its cephalic margin may serve as a good line of division between the prosencephal and diencephal. The infundibulum is well developed and is relatively wide at its origin at the chiasma; it grows still wider in its course toward the oblongata; its caudal margin is convex except at the meson where is present an indentation caused by the intrusion of the hypophysis.

The mesencephal is almost entirely concealed by the infundibulum. The metencephal is widest at its cephalic extremity and tapers gradually into the myel.

MENINGES.

In the Teleosts the meninges are represented by pia and dura only, the former, perhaps, also including the arachnoid,

according to Sagemehl (46). He proposes to call the pia or its homologue the primary vascular membrane. Fulliquet (12) accepts this view for the conditions found in the Protopterus. Burckhardt (3), in his studies of the Protopterus, describes the pia and arachnoid, but has nothing to say of the dura. He discovers a structure along each side of the myel homologous to the *ligamentum dentatum* of higher forms. He has also found it in the sturgeon, but divided, one part being nearer the dorsal root, the other nearer the ventral. He considers it a differentiation of the pia, and supportive or protective in function on account of the great lateral mobility of these forms. Mrs. Gage (13) finds all three of these membranes represented in the *Diemyctylus*. The dura is characterized by numerous large pigment cells. It lines the cranial cavity, sending off an almost complete investment for the supraplexus, and a partial one for the hypophysis. It follows out the cranial nerves, and surrounds their ganglia. She finds that the pia does not always follow the outline of the brain closely, but sometimes lies nearer the dura, probably an effect of hardening. It forms a single layer between the hemicerebrums, and contains blood-vessels; it separates the hypophysis from the infundibulum, and forms a continuous sheet over the epiphysis. The arachnoid is represented as a spongework, formed of connective tissue cells.

In the *Desmognathus* the conditions are substantially the same as in *Diemyctylus*. The dura seems to lose its pigment cells along the floor of the cranium. The pia is quite closely adherent to the nervous substance. It consists of a thin layer of cells the even contour of which is occasionally broken by the slight elevation of their nuclei. In or upon this layer are the blood-vessels. A complete investment or separation of the hypophysis from the infundibulum could not be satisfactorily demonstrated. Numerous pigment cells are found in the metaplexus, as well as in the dura.

The arachnoid is represented between the two membranes just described by loose trabeculæ of connective tissue. It entirely disappears along the base or ventral aspect of the brain and myel, on account of the meeting of the pia and dura along

this region. Its trabecular character also suggests the probability of the presence of lymph.

BLOOD SUPPLY.

The difficulties of tracing out the circulation in such a small animal, where injection is impossible, are obvious enough. The general description of the blood-vessels of the cerebro-spinal nervous system of the urodeles, as demonstrated by Schöbl (50), in *Salamandra maculosa*, *Triton*, *Proteus*, *Amblystoma*, and *Necturus*, it is believed, will, in all essentials, correspond with the conditions incidentally observed in the *Desmognathus*. The substance of the brain and myel contains neither arteries nor veins; these lie upon the surface; they are very much branched, and send capillary loops into the interior. These vascular loops have also been noticed by Mrs. Gage. Schöbl describes and figures the loops as continuing nearly to the endymal line. In the *Desmognathus* most of these loops stop at the margin of the entocinerea, but occasionally they have been seen to enter it a greater or less distance. The carotid arteries, on entering the cranial cavity, divide into a cephalic and caudal ramus; the former passes along the side of the mesencephal to the optic nerve, where it bifurcates; one branch, the inferior external, passes along the latero-ventral aspect of the hemicerebrum to the olfactory nerve, and gives off numerous branches to the ventral and lateral aspects; the other — the superior internal artery — supplies the dorsal surface of the cerebrum, passing along its dorso-mesal edge, and giving off a branch to the supraplexus. Occasionally, on the ventral aspect, a strongly-developed branch from the inferior external artery meets its opposite, and forms a cephalic communicating artery.

The caudal rami bend meso-caudad and anastomose ventral to the hypophysis, thus forming a caudal communicating artery from which springs the spinal artery by means of two or three roots. The posterior communicating artery gives off two branches, each one supplying the ventral, lateral, and, to some extent, the dorsal aspects of the mesencephal; still another

pair is given off to supply the hypophysis. The spinal artery sends off branches, at intervals, which supply the ventral and lateral aspects of the myel. Most of the capillary loops here penetrate the substance from the sides, few from the dorsal or ventral surfaces.

Concerning the venous system, Schöbl finds one paired and two unpaired venous plexuses. The supraplexus is one of the latter, and lies between the hemicerebrums, forming, in part, the roof of the diacœle. Three veins usually join the plexus at its cephalic angle; the middle one of the three is derived from the pia of the cerebrum, while the two lateral ones convey the blood from the lateral, wide-meshed, venous nets interlacing the dura with the plexus. From the caudal angles of the supraplexus there goes out on each side, around the caudal ends of the cerebrum, a vein which connects the supraplexus with the paired lateral plexuses lying on either side of the mesencephal. The lateral plexuses receive their blood from the supraplexus and from a lateral vein which, with its fellow, forms a loop around the margin of the cerebrum. In the region of the cerebellum they continue as the jugular veins. The metaplexus covers the cerebellum and metacœle; it receives its blood from the region of the myel, and sends it into the jugular veins.

There are no plexuses on the ventral aspect. A longitudinal vein, lying between the hemicerebrums, passes caudad, and, in the region of the mesencephal, divides to form an irregular circle, or pentagon, from the latero-caudal angle of which a vein connects on each side with the lateral plexus. There is found quite constantly, just caudad of the hypophysis, a transverse vein connecting the two lateral plexuses. The small venous branches of the cephalic portion of the myel collect into two lateral trunks, and empty into the metaplexus. Farther caudad, these branches unite to form the spinal vein, which passes caudad along the dorsal surface of the myel.

Rex (43), besides a few minor details, criticizes Schöbl's account on the ground that it deals more with the surface circulation, and that the figures are schematic.

CRANIAL NERVES.

The size of the nerves is remarkably large, disproportionately so for so small a brain and this in connection with their rather blunt terminations seems to be an evidence of the persistence of certain embryonic features. The following description deals only with the apparent origin of the nerve roots and makes no attempt to trace out the peripheral distribution. The study is based upon decalcified specimens verified to some extent by the examination of sections.

I. The olfactory nerve is joined by a single root to the cephalo-lateral angle of the hemicerebrum, agreeing in this respect more closely with the condition found by Kingsley (19) in a larval *Amphiuma* rather than with that found by Mrs. Gage (13) and Burekhardt (2) in *Diemyctylus* and *Triton* respectively. It is a little nearer to the ventral than the dorsal aspect and soon divides into a dorsal and ventral branch, which again subdivides to supply the nasal capsule. Jacobson's organ receives its innervation from a branch of the ophthalmic.

II. The optic nerve contains a distinct central axis of cells. Mrs. Gage (13) found that in *Diemyctylus* there were no fibers on the dorsal side of this nerve at its attachment with the brain. The chiasma is not very distinct superficially, as it has apparently sunk into the brain substance and protrudes very slightly or not at all from the surface.

III. The oculo-motor nerve is fairly well developed and seems to arise from the usual point in the mesencephal.

IV. The trochlearis I failed to demonstrate, though commonly present in the Amphibia; Kingsley (19) likewise failed to find it in a larval *Amphiuma*.

V. The trigeminus arises from the cephalo-lateral angle of the oblongata. It is quite closely applied to the mesencephal on each side; at nearly the level of the caudal tip of the cerebrum it leaves the cranium. In the foramen is the Gasserian ganglion. Three branches leave it, the ophthalmic, maxillary, and mandibular. The former passes along the ectal surface of the cranium and dorsad of the optic to the olfactory

nerve where it subdivides, one branch going to Jacobson's organ, the other to parts cephalad of the eye. The maxillary branch joins the ganglion a little dorsal to the mandibular, both extend laterally, but were not traced for any length.

VI. The abducens arises from the ventral aspect about midway between the origins of the eighth and ninth. It is a very slender nerve and passes off toward the Gasserian ganglion, but a distinct union with it was not demonstrated. Kingsley (19) did not find it in a larval *Amphiuma*. Although very minute and easy to escape detection it is generally believed to exist in *Amphibia*. Mrs. Gage (13) found it in the adult *Diemyctylus*, but only a trace of it in the larva.

VII, VIII. The facial and auditory nerves are apparently fused into one trunk. The combined root is just caudal to and in line with the fifth. It would appear that the portion corresponding to the eighth is slightly cephalo-ventral to the caudal branch of the seventh. From the common trunk there passes a good sized branch toward the dorsal surface of the Gasserian ganglion, but I failed to establish an actual union; it probably corresponds to the palatine branch. The other, the hyomandibular, arches latero-caudad. In the angle of divergence between these two branches and more to the ventral side of the trunk lies the auditory nerve joining with the ear capsule by two or possibly three divisions. The eighth nerve is ordinarily represented as extending caudad of the seventh, and it seems quite likely that its more forward position in this form may be due to a cephalo-caudal compression of the head parts. There are other features connected with the brain which seem to warrant this view.

IX, X, XI. The glossopharyngeus, vagus, and spinal accessory arise by separate roots, that of the tenth being the largest and forming apparently the chief part of the trunk of which the other two are simply reinforcements. All three arise about in a line and nearer the dorsal than ventral margin. A short distance out there is a ganglionic enlargement. From the ganglion three branches are given off; one passing cephalad connecting with the seventh, the other laterad, and the third and largest caudad.

XII. The hypoglossus is included under the cranial nerves with some misgiving. The consensus of opinion seems to be against it in the *Amphibia*. In the *Desmognathus* a small ventral nerve arises some distance caudal to the vagus outside the cranial cavity and is possibly nothing more than the ventral root of the first pair of the spinal nerves.

Rolleston (45) states that there is no spinal accessory nerve in *Amphibia* and that the area supplied by the hypoglossal is supplied by the first spinal nerve in the majority.

GENERAL MORPHOLOGY.

It is generally held by working neurologists that the adult vertebrate brain shows evidence of differentiation into segments not necessarily correlated numerically with those found in the embryo.

In forms above the lancelet, five "definitive" encephalic segments are commonly recognized: the prosencephal (including the olfactory lobes), the diencephal, the mesencephal, ependecephal, and metencephal. Whether the olfactory portion deserves setting apart as a separate segment under the name of rhinencephal is a matter still under discussion. It is a question for embryology and comparative neurology to determine. Wilder (58) has called attention to the potential triplicity of these five segments which, although primarily mesal and simple, present at some time and in some vertebrate, ontogenetically or phylogenetically a threefold or tripartite condition. In the prosencephal there is the mesal aula with its lateral extensions, the paracœles or lateral ventricles. The suggestion is offered by him that a portion of the mesal aula may be considered as belonging to the rhinocœles, thus bringing the rhinencephal in line with the other segments. In the diencephal, the diacœle and its optic recesses — remnants of the optic vesicles; in the mesencephal, notably in birds, the aqueduct with its lateral extensions into the gemina; for the ependecephal Wilder gives the epicœle for the mesal part and the lateral recesses or parepicœles for the lateral; while the metencephal, except in the *Torpedo*, *Catostomus*, and some other

forms where it is excessively developed, is apparently an exception to the tripartite rule.

In the *Amphibia* and in the early stages of some other forms the cerebellum is nothing more than a commissure and is an azygous or mesal part as is the epiphysis and infundibulum, while in the Hag fishes it is quite likely there is no cerebellum at all. There is moreover below mammals no pons to help us over the difficulty of finding a dividing line between the ependecephal and metencephal or differentiating a pre- from a post-oblongata. The infundibulum and the parts connected with it in many animals show this triple arrangement which, with its probable intimate relation to the morphological front of the head in the embryo, has led some to believe that this region might also claim the distinction of a separate segment. Kupffer (24) has proposed the term *hypencephalon*; the same author, contrary to common usage and all precedent, applied the term ependecephalon to the great brain or prosencephal.

Physiologically there is more or less of a dual connection of the brain throughout all of these segments and from the standpoint of a duplicity or bilaterality of the parts there are morphological as well as physiological grounds for admitting the rhinencephal as a segment.

In the lancelet the olfactory is the most strongly differentiated portion of the neuraxis; but the asymmetry and the absence of either duplicity or triplicity of this region render this animal an exception to the general vertebrate rule. Mrs. Gage (13) finds that in the embryo of *Diemictylus* "there are two portions of the forebrain—one associated with the developing olfactory nerves, the other lying next the diencephal—with a large common cavity."

The cephalic part of the cavity belongs to the olfactory region and the caudal to the cerebrum, a condition approximately retained in the lamprey, and she therefore concludes that the rhinencephal is quite legitimately entitled to a share of the aula as a mesal cavity, and consequently possessing a tripartite arrangement.

Steiner (52) after experiments upon the different segments of the brain of some of the lower vertebrates, especially the

sharks, found that he could obtain the same results by separating the olfactory organ from its central connection as by removing the entire forebrain; from this he concludes that the prosencephal of the shark consists of nothing else but an olfactory center, and since the prosencephal of every vertebrate is homologous it further follows that the prosencephal of the vertebrate has developed phylogenetically out of the olfactory organs. A comprehensive definition of the brain is out of the question, but after his numerous experiments and observations he constructs the following formula: The brain is the common center of movement in connection with the function of at least one of the higher sense organs.

Indeed phylogeny, pointing to the lancelet, lamprey, and elasmobranchs, seems to suggest that the olfactory was the primitive sense as well as segment, and it may be true as suggested by Wilder (57) "the prevailing idea that the olfactory lobes are mere appendages of the cerebrum is nearly the reverse of the truth."

Rhinencephal.—It is a curious fact that the term *rhinencephale* as first used by Geoffrey St.-Hilaire and by Robin, had no reference whatsoever to the brain, but was applied to a genus of unocular monsters characterized by the conversion of the nose into a sort of proboscis. Owen applied it to the brain believing that the presence of a cavity in the olfactory bulb entitled it to segmental distinction. Later, Sir William Turner has used the term in a more unsegmental sense. He has proposed calling all that portion rhinencephal which is separated from the pallium by the olfactory fissure. Kupffer (24) has made the important discovery that there exists a dorsal neuropore in the embryo of *Acipenser* homologous to the condition found in the lancelet. In the neural tube, as it draws away from the ectoderm at this point, there remains a slight projection which he calls the *lobus olfactorius impar* constituting the very front of the brain. He has as yet failed to demonstrate its existence in *Ammocetes*. Rabl-Rückhard (41) has found a mesal recess in the terma of an *Acanthias* embryo believed to be homologous with the conditions found by Kupffer. Whether a similar structure can be demonstrated in all vertebrates is

open to some question. The conclusion is that the olfactory segment arose from a single median plate. His (18) proposes the term *angulus terminalis* believing that it has no connection with the olfactory region. Wilder (58) has found in the human brain "a subtriangular depression between the precommissure and the two fornicolumns" which he calls the *aulic recess*. From its mesal position in the terma and general form it is quite suggestive of being a remnant of the primitive conditions described by Kupffer, Rabl-Rückhard, and His in lower forms, if the relations of the surrounding parts can be homologized.

In the *Desmognathus* there is no very distinct ectal separation of this segment from the prosencephal. The olfactory nerve is attached along the ventro-lateral portion and may be correlated in some way with the greater lateral than mesal length of this segment. Along this shorter mesal margin there is a condensed aggregation of cells forming an ectocinerea; this occurs nowhere else in the whole brain, except perhaps at the habenæ; there are also numerous cells scattered around the attachment of the olfactory nerve. When the brain is divided into frontal sections (Fig. 6) a very decided angle is noticeable at about the middle of the length of the cavity of the cerebrum; that portion from the angle cephalad is believed to have grown away from its original direct communication with the aula as noted by Mrs. Gage in *Diemyctylus* larvæ and to be the rhino-cœle or cavity proper of the rhinencephal.

Prosencephal.—The prosencephal merges out of the preceding segment, growing wider caudad as the hemicerebrums divaricate. The supraplexus is located at this angle of divergence and on its entrance into the cavity divides into two main branches (Fig. 3) the auliplexus and the diaplexus; the former sends off a branch into the cavity of the infundibulum before dividing to enter the paracœles (lateral ventricles). The diaplexus sends off a branch toward the epiphysis and then passes caudad as far as the cerebellum.

The terma in *Desmognathus* according to my interpretation extends from the chiasma cephalad and then dorsad until it reaches the supraplexus; the dorsal portion is membranous — a tela, and shuts off all communication of the mesal cavity with

the intercerebral fissure; the bulkier and principal portion forms the floor of the mesal cavity from the chiasma to where the aula sends off the paracœles, and gradually merges into the tela. It includes the precommissure and the callosum.

The question as to the existence of a callosum in the lower vertebrates is a very perplexing one, and perhaps as yet has not been conclusively answered. In establishing homologies it seems to me it is well to keep in mind not only the facts of relation and distribution as they are more or less clearly shown in the higher forms, but to consider the development of the various stages and as far as possible correlate phylogeny with ontogeny. Osborn (36) has conducted a most careful and searching phylogenetic investigation of this subject and concludes that the callosum exists in the various vertebrate classes from "fishes" up. Symington (54) has investigated this region in the marsupials and monotremes and in these low mammalian forms finds the precommissure strongly developed and a hippocampal commissure which has ordinarily been taken for the callosum. In the absence of this commissure (callosum) his conclusions concur with the earlier opinions of Owen, and are opposed to those of Flower and some others.

Since the callosum reaches its highest development in man and its size is doubtless correlated with that of the cerebral hemispheres it is, therefore, only proper that the conditions found here where the morphology is clear and unmistakable should be taken as the basis for homology. In man it forms nearly or entirely the roof of the paracœles or lateral ventricles. It is composed largely of commissural association fibers joining different areas of the two hemispheres, and contains likewise numerous collaterals from the cortical projection fibers. It is unquestionably the fact that, in some of the lower forms, notably the *Amphibia*, the so called callosum is, instead of a roof of a cavity, apparently a portion of the floor of one—the aula or ventriculus communis, and instead of being in front and above (cephalo-dorsad) of the porta (foramen of Monro) it is in some forms under and behind it (ventro-caudad). Some of the more recent methods, especially Golgi's silver nitrate method, do not seem to have been very extensively employed in

the neurology of the *Amphibia*, and the exact origin and individual relations of the fibers is not very definitely known. From an embryological standpoint these morphological differences need not be of much moment.

Marchand (28) in a series of beautiful figures has shown the development of these commissures in the human brain as seen in mesal section, the first appearance being a fibrous thickening of the terma. This is the *proton* or *anlage* of the precommissure, and might at the same time be considered to contain the potential rudiments of the fornix and callosum, for as development goes on, this area as seen from the mesal aspect soon differentiates into a hook-shaped mass by the addition of new fibers due to the increased growth of certain of the cerebral parts. It is quite inconceivable that the fibers forming the callosum should jump across such a gap as the intercerebral fissure; the presence of the indusium or vestigial cortex found on the callosum in the adult mammals is pretty strong evidence that the new fibers are laid against or insinuate themselves into this original bundle, and in this way the callosum elongates cephalad and becomes thicker proportionately to the development of the cerebrum.

In the *Desmognathus*, where the brain is so elongated and the terma forms so large a proportion of the floor, it is at first sight rather puzzling to determine the homologies of these commissures. The morphological relations are as obscure as they possibly could be; the precommissure apparently arises from the floor as a short column of transverse fibers, as seen in mesal section, bearing on top of it a smaller bundle, the callosum, or as I interpret it, an undifferentiated forni-callosum, since many of the fibers seem to correspond with those as described by others for the hippocampal commissure passing to the caudal ends of the hemispheres, while others, the callosal fibers, are confined more to the mesal walls and help to a slight extent in forming the roof of the paracœles in this region. The *aula* lies not only in front (cephalad) of these commissures, but to some extent lies under or ventral to them. If now, instead of stopping at this stage, the growth of the cerebrum should go on in a dorso-caudal direc-

tion analogous to that of higher forms, then would the terma and its commissures pass or bend in a cephalo-dorsal direction through an arc of ninety degrees and the morphological relations would approximate to those of higher vertebrates.

The difficulty of homologizing these parts has been largely due to the inadequate or inaccurate limitations put upon the boundaries of the terma. To homologize with higher forms and with a due regard for the morphological front or cephalic end of the brain, it seems to me its most ventral or caudal boundary should be at the chiasma and the dorsal or cephalic boundary where it meets the supraplexus. Wilder (58), with the mammalian brain as a basis, has proposed the terms proso-terma for that portion dorsal to the precommissure, and dia-terma for the remaining portion which he also regards as properly belonging to the floor. In the absence of any special line of demarcation in the terma itself or of the aula, such a division seems unnecessary, for there is no apparent change in the morphology except that the area allotted to the aula is somewhat increased. These commissures are all developed in the terma and assume certain relations to the other parts according to the development of the cerebrum. When the cerebrum has developed to such an extent as to overlap the diencephal and mesencephal, it must obviously cause the terma to bend in a dorso-caudal direction and markedly change the relations of the parts in this region; so that in the *Amphibia*, where a considerable portion of the terma is ventral, the commissures seem to spring from the floor; but if the terma be bent upward and backward (dorso-caudad), allowing for a corresponding and perhaps different rate of change for the cavities, the homology would be much more evident. Figs. 20-24 represent the position and relations of these parts in the *Desmognathus*, *Cryptobranchius*, frog, turtle, and bird. These forms make a good series phylogenetically for the illustration of this point and for the exemplification of what has just been said.

Osborn has discovered that the callosum in *Necturus* is entirely separated from the precommissure by a fold of the plexus and he states that a similar condition exists in the

Proteus. Herrick has confirmed this in the *Necturus* and finds it a matter of congratulation, because the plexus being a diverticle of the roof and separating the commissures in this way, he recognizes "of necessity that the commissure is morphologically dorsal" and belongs to the roof and not to the floor of the ventricle. I cannot but feel that this arrangement in *Necturus* is of but little or no morphological importance, and is only an exaggerated variation of the conditions found in allied forms. We find that in some *Amphibia* these commissures are practically one, while in others they may have grown more or less apart, but in no case that I know of to such an extent as is described for the adult *Necturus*. In all these other forms then, the callosum arises at the same time or as a part of the precommissure from the floor, or more properly the terma, of the ventricle. A study of a series of transections through the head of a young *Necturus* 38 millimeters in length, shows that there is no such separation of the callosum as is described for the adult; there are some cells interpolated between it and the precommissure as in the *Desmognathus*, but not the least indication of a separation by means of a cavity. Observations upon the brain of an adult *Necturus* cut into sagittal sections likewise showed a non-separation of these commissures except by a simple cellular layer. Although the plexus may have arisen as a diverticle of the roof and later interpolated one of its branches between these commissures, this fact need not necessarily make the commissure a part of the roof, for we might on similar grounds call the dorsal or caudal wall of the infundibulum a part of the roof, because in some instances a branch of the plexus extends into its cavity.

Herrick (17), Pl. XV, Fig. 5, and Pl. XVIII, Fig. 5, shows in the *Necturus*, as the callosum, a slight projection caudad of the mesal walls of the cerebrum and represents fibers appearing from it in these same mesal walls or intra-ventricular lobes. In a later paper (The Callosum and Hippocampus in Marsupials and Lower Brains, *Jour. Comp. Neurology*, III, pp. 176-182. 2 plates, 1893), he reiterates this statement, but also adds that "it might still be considered possible that callosal elements were bound up in the larger hippocampal commissure." Mrs.

Gage (13) has noticed a similar projection in the *Diemyctylus* and *Amia* and has called it the crista, appropriating a name given by Wilder (56) for a similarly appearing object discovered by him on the fornix just dorsal to the precommissure in the adult cat and sheep and in the human embryo. In the *Desmognathus*, this part contains no fibers and appears to be nothing more than an intrusion of the membrane, the pia, covered only by a layer of endymal cells; in one specimen there was a distinct loop here. She also proposes the term *callosal eminences* for what Herrick figures as the intra-ventricular lobes. The terms crista and callosal eminence are employed in this article for convenience and to avoid inflicting new terms, and not because it is believed a strict homology can be established.

At least two conditions should be considered with regard to the phylogeny of the callosum; its size relative to that of the cerebrum and its relation to the amount of the cerebral cortex. In the birds it is weakly represented, although the cerebrum is of considerable size. It is proportionately much less developed in this class than in the *Amphibia*. Osborn attributes its small size to the thinness of the mesal wall. It cannot be due to the insufficiency of cortex, as this greatly exceeds that of the *Amphibia*, and the birds, therefore, seem to be an exception to these two important factors.

A brief survey would seem to indicate that the precommissure is the first in point of development and usefulness, and that the callosal and hippocampal fibres practically arise simultaneously, and are of equal importance in these low forms. Regarding these hippocampal fibres as the precursors of what is commonly known as the fornix, it is worth noting that in some mammalian brains, where the callosum has not developed, there were only the precommissure and fornix found, and in some perhaps not even the fornix; while in no case within the writer's knowledge has a brain been described with the callosum well developed and either or both of the other commissures absent.

The rima, or transverse fissure, does not exist in the *Amphibia*. In mammals, its general direction is caudo-lateral.

Wilder (57) has described it in the *Ceratodus*, a dipnoan, but finds that its direction is toward the cephalic end of the brain, which may be due to the ventral position of the cerebrum. With a possible exception of a few reptiles, the rima does not distinctly appear again until the birds, where it is but slightly developed. In mammals it reaches its highest development, extending from the porta to near the tip of the temporal lobe; and the *Amphibia*, birds, and mammals seem to form a series, showing the essential stages of the development of this part.

In the relatively long and narrow amphibian brain, with the cavities about all on the same level, and with the supraplexus dipping down through the roof, there is little need of a rima; but in the case of the birds, where there is more compactness, where many of the parts have widened and overlapped certain of the other parts, radical changes must take place with regard to the plexus and its relations. From the position of the plexus and of the rima and its degree of development in the three forms above mentioned, a most natural inference would be that the rima is caused by the plexus beginning at the porta and cutting its way along the floor of the paracœle toward the side of each hemiserebrum; but the plexus, covered by its lining epithelium, — the endyma, — effectually shuts off any natural communication between the two, and the porta is entirely circumscribed by the endyma. It is not improbable that several conditions are concerned in bringing about the rima, that not only the lateral growth of the parts, but the cranial flexure and great development of the callosum are likewise involved.

Paraphysis. — The paraphysis is an outgrowth in the roof of the brain cavity, and it is variously said to belong to the prosencephal and diencephal. It is enclosed in the supraplexus, and has been suggested as having some relation to the neuropore. Eycleshymer (10) has described it in *Amblystoma* larvæ up to 14 mm., when the proximal part of the cavity becomes obliterated. Mrs. Gage, however, found it well developed in the adult *Diemyctylus*, and its cavity, though somewhat constricted, still continuous with the brain cavities. In the *Desmognathus* it has been more easily seen in frontal, or

horizontal, sections. Various functions have been ascribed to this part. It has been thought to be concerned in vision and audition. Mrs. Gage has also suggested that it may be a trophic organ, concerned in the nourishment of the brain, since it appears before the plexuses in a region which "has need of a means of repairing waste."

Diencephal. — This segment merges into the mesencephal with no ectal sign of differentiation. The cavity (diacœle) is narrow, but has a greater dorso-ventral diameter than has any other cavity of the brain. On each side of the wall are two sulci, beginning at the portas and extending in a caudal direction. Both are rather indistinct at their origin, but soon become deeper; the ventral one extends, somewhat obliquely, toward the base, and expands into the infundibulum; the dorsal sulcus (the sulcus of Monro or the aulix of Wilder [58]) takes a more direct course, and expands into a fairly-wide mesocœle. The infundibulum is, relatively, very large, and, from the ventral aspect, almost entirely obscures the mesencephal. Its cavity is not high, dorso-ventrally, but has considerable lateral extension. The hypophysis is quite closely applied to the caudal end of the infundibulum. It appears somewhat glandular in structure, and is highly vascular. Immediately in front of the chiasma is a slight lateral extension of the diacœle along each optic nerve, representing the preoptic recess. Considerable interest centers about this region on account of its connection with the morphological front of the brain. Different anatomists have taken different standards. If the axis pass along the roof of the neural tube, then it will fall upon the summit of the terma; if it pass midway between the floor and the roof, it will terminate at the preoptic recess; if the floor be chosen, then the axis will end in the infundibulum. His favors the last view.

In the roof of the diencephal, and just caudal to the supraplexus, are the habenæ; they are not conspicuous on the surface of the brain, being overlapped by a caudal projection of the plexus. In transections, the left habena seems to be larger than the right (Fig. 28). The supracommissure is quite well developed, and easily seen in frontal or transections. The

epiphysis in the adult is quite small, appearing like a very much depressed button or pad, lying just caudal to the supra-commissure. There is usually no trace of a lumen further than a small central accumulation of endymal cells. In a 17-mm. larva there was a distinct cavity, but on account of the inaccessibility of earlier stages than this, I was not able to determine an actual continuity of its cavity with the diacoel. The postcommissure is caudal to the epiphysis, and, as has been suggested by Osborn (37), may be regarded as a line of demarcation between the diencephal and mesencephal.

Mesencephal.— This segment is subspherical in outline, as seen from the dorsal aspect; the cavity enlarges laterally, but there are no indications of a division into the optic lobes, so characteristic of the *Anura*. The entocinerea of the roof is well developed; in some young specimens examined, the cells extended to the ectal surface at the dorsal median line. In older specimens there is a considerable layer of alba between, in which are scattered a few nerve cells. The cinerea, however, is divisible into two nearly equal layers by the interpolation of a thin stratum of alba; on the meson it is scarcely perceptible, but laterally it is well developed, and extends from the post-commissure nearly to the cerebellum, and is possibly significant as separating the cells into an ecto- and entocinerea. Osborn has found eight different layers in this region in *Rana*.

Epencephal.— The cerebellum appears to be the most differentiated part of this segment. It is not at all apparent from ectal observation, but lies bent up under the caudal margin of the mesencephal. There are a few cells along its cephalic face; it is constricted at the meson, is made up chiefly of fibers, and is undoubtedly commissural in function as stated by Osborn (38). He discriminates between fine and coarse fibers; the former he believes to be in part decussating tracts of the auditory nerve, and the latter non-decussating descending tracts of the trigeminus nerve. Some support is given to the view of connection with the auditory nerve by the observations of Köppen and Ahlborn. There is nothing in the oblongata to differentiate any portion of it as belonging to this segment, unless it be the origins of the fifth, sixth, seventh, and eighth nerves.

Metencephal.—From this segment are given off the ninth, tenth, and eleventh cranial nerves. It tapers gradually into the myel. The cavity expands in the region of the cerebellum and these lateral wings may possibly be analogous to the lateral recesses or parepicœles of higher forms. In its caudal direction the cavity becomes quite narrow and high, but slopes quite abruptly to form the myelocœle. The metaplexus, the roof of the metacœle, is quite compact and quadrangular in outline. It seems to shut off all connection between the cavity and the outside, and I was not able to find any trace of a metapore or foramen of Magendie as has been described by Mrs. Gage in this region of the *Diemyctylus*.

Myel.—The myel is a subcylindrical cord presenting very slight enlargements in the cervical and sacral regions. At the *foramen magnum* there is quite a marked constriction. There is scarcely an indication of a dorsal furrow, while that of the ventral side is very noticeable and contains quite a good-sized blood-vessel. There is also a dorsal vessel—the spinal vein, but much smaller in size. The membranes have about the same relation as with the brain. There is a greater separation of the dura and pia, and the arachnoid is therefore more fully developed. In the larva the spinal canal is not as nearly filled up by the cord as in the adult and the meningeal relations can be studied to much greater advantage.

The spinal ganglia are very large, their dorso-ventral diameter being much greater than the lateral. They are situated outside of the spinal canal in the intervertebral foramina. They are enveloped in dura and lie close up against the muscle segments. The majority of the cells composing these ganglia are large and suggest an analogy to the “periganglionic glands” found in the frog, although no milky fluid nor any evidence of calcareous matter has been found. The ganglia are attached chiefly to the dorsal roots but embrace the distal portion of the ventral roots as well. In the frog the periganglionic glands have been found on the ganglia of all spinal nerves as well as the Gasserian ganglion of the trigeminus nerve.

The existence of a *ligamentum dentatum* homologous to that found in higher forms and as described by Burckhardt for

Protopterus was demonstrated in the *Desmognathus*. The specimens showed what appeared to be a slight thickening of the pia between the dorsal and ventral roots; while in a small larva of 17 mm. there appeared a cord, Fig. 18, similar in size but nearer the ventral than dorsal root, connecting the ventro-lateral angle of the myel with the dura or ganglion, and it seems quite likely that this cord which has quite a semblance to a lateral root is functionally a *ligamentum dentatum* excessively developed in order to support the myel, which as yet does not nearly fill up the spinal canal. When the canal becomes more completely filled the ligament becomes reduced to a much smaller size and loses its peripheral attachment. Figs. 37, 38, 54, 57. The ventral root leaves the myel not far from the ventral furrow, the dorsal root enters at about the dorso-lateral angle of the myel.

In transection the lateral diameter of the myel is greater than the dorso-ventral; the myelocœle is small but distinct and shows no traces of cilia, as indeed did none of the brain cavities. Numerous cells, from five to seven layers deep, surround the cavity, forming an entocinerea; this ental column of cinerea is preserved throughout the whole neuraxis and is in marked contrast to the condition found in mammals where the brain is characterized by a strong development of ectocinerea. Of these layers of cells those most dorsal approach most nearly to the periphery. The dorsal and ventral horns are quite well marked; the latter are relatively broad and do not extend as near the surface as do the dorsal. The cells are not confined to the cinerea, many are found scattered irregularly through the alba. There is, perhaps, some appearance of grouping at the ventro-lateral angles of the cord and this may possibly have some significance in connection with the close proximity of the ventral nerve root.

Klaussner (20) differentiates the cinerea of the myel into zones, the most ental of which is composed of epithelial cells from five to six cells deep; the middle zone shows a distinct fibrous structure and consists of fine fiber tracts and networks; the ectal zone lies next to the alba and is characterized by the appearance of what he considers true nerve cells.

The largest of these lie in the ventro-lateral angle of the gray mass. Certain round cells or granules are present in the dorsal horns; between these and the epithelial and the large nerve cells all kinds of transitions are found. He was not able to demonstrate with satisfactory clearness a connection between the fibers of the ventral roots and the large nerve cells, and concludes that the central epithelial cells have a true nervous function, basing his view especially on the fact that he found fiber tracts coming from these cells into the ventral commissure and dorsal roots.

Roller (44) was perhaps the first to allude to the nervous nature of these epithelial cells. He saw fibers going from them to a group of nerve cells—the nidus of the glossopharyngeal nerve.

GENERAL HISTOLOGY.

The retention of so many of the primitive and simple conditions in the nervous system of a group so advanced as the Amphibia renders it a very desirable class for study preparatory to more specialized forms. The amphibian brain is regarded by Edinger as the simplest in the vertebrate series. Here, it is quite likely, will be found the key that will unlock the door of many morphological and histological problems. A simplicity of the general morphology of a part does not necessarily involve a corresponding simplicity of its histology. Perhaps these two conditions are exceptionally simplified in the *Amphibia* for the neurocytes (nerve cells), and their relations retain embryonic features to a remarkable extent when treated by ordinary methods. The silver method reveals a more complicated state of affairs, but as compared with similar structures in other forms they are still relatively simple. The texture of the neuraxis varies greatly even in closely associated genera, and should be taken into account in "fixing" the material. Experimentation is quite necessary, particularly with Golgi preparations. With aquatic forms the tissue seems to be less dense than with terrestrial.

As has been stated by Oyarzun (39), much of the work done on the amphibian nervous system has been in tracing out fiber

tracts while the cells have been practically ignored. The older methods are poorly adapted for demonstrating cellular morphology, and the apparent simplicity of the cells did not merit a very extended description. Reissner, Stieda, Bellonci, Osborn, and several others have contributed very largely to the knowledge of the neurology of this group, considering the methods at their disposal. Oyarzun (39), Lavdowsky (25), and Sclavunos (51) by the application of the silver nitrate method have added information that is new and valuable. Oyarzun confined his investigation to the cerebrum of the frog, salamander, and *Triton*, and obtained essentially the same results in them all. He distinguishes two quite different kinds of cells: first, the endymal or those lining the cavities of the brain, which send off a process toward the periphery; this subdivides and forms a dense network. He concludes that this net or fibrous interlacement forms the supporting tissue or chief mass of the molecular layer. The second kind of cell is found at any level in the cell layer, but never directly bounding the cavities like the endymal cells. They are multipolar and send out numerous processes in an ental and tangential direction, while often from the ental side of the cell a smooth process was noted passing mostly in an ento-caudal direction (the axis-cylinder). A general analogy is noted between the cells and their processes and supporting substance, and the conditions existing in the early embryonic stages.

Lavdowsky (25) treats of the myel of certain mammals and *Anura*, especially of the neuroglia and nerve cells. In the myel of the frog he identified four kinds of glia cells: First, the wedge-shaped endymal cells, the blunt end being next to the cavity and the long peripheral process projecting from the opposite end. Second, large branching cells, which resembled pigment cells, sending off processes peripherally through the alba to connect with the "pin" fibers of Stieda from the pia. The third form are the so-called "pin" fibers which according to Lavdowsky's view are not fibers at all, but cells drawn out into the appearance of fibers. The fourth kind is represented by a conglomerate of small cells situated just ventral to the myelocœle. With regard to the nerve cells, he finds no essential

difference between the axis-cylinder and protoplasmic processes, basing his opinion not only upon silvered preparations, but upon sections prepared by other methods and upon teased specimens.

From a series of longitudinal and transections he finds: (1) Cells of the ventral cornua and mesal portion of the myel sending some of their processes into the ventral or anterior commissure and others into the ventral and lateral columns as well as into the ventral roots. (2) Cells of the same horns which lie close up to the lateral columns and send off processes which pass along the inner side of the lateral and ventral columns and bend around in these columns into the ventral commissure and root. (3) Cells of the mesal part whose processes pass off into the dorsal cornua, dorsal columns, and both commissures. (4) Cells of the ventral and mesal part with processes entering into the dorsal and ventral roots. (5) Cells of the dorsal cornua whose processes go over into the dorsal nerve roots and dorsal columns. (6) Cells lying beside the dorsal horns which send processes partly into the dorsal roots and partly into the dorsal columns.

In the myel of the toad he found (7) some cells sending their processes into the dorsal column and others into the ventral column. (8) Cells of large size lying on the inner side of the white columns sending their processes to the ventral columns and roots, and others to the dorsal, and still others to the lateral columns. (9) Small cells with relatively delicate processes forming partly the dorsal and ventral columns and partly the commissural fibers and nerve net of the gray substance.

In sagittal sections of the toad myel he noted (10) cells with processes bending around into the dorsal and ventral columns and continuing undivided, or dividing into a cephalic or caudal branch. (11) Cells whose processes passed farther into the gray substance, and on the one hand formed the gray nerve net and on the other enter into the white columns. In frontal sections he finds (12) cells whose processes in part run in toward the central canal and in part to the lateral columns where they may divide, or having divided earlier, send their

branches into the lateral columns. Concerning the dendritic processes of the nerve cells, he concludes that they, after numerous divisions, pass into the nerve-conducting paths as the axis-cylinders themselves.

Golgi (15) criticises the article severely, especially Lavdowsky's inability to find a distinct axis-cylinder process.

The investigation of Sclavunos (51) was likewise confined to the myel and was based upon the larvæ of *Salamandra maculata* and *Siredon*. Especial attention was given to the dorsal roots. He was unable to find fibers passing through the ganglion and continuing undivided in the myel as has been described by Lenhossék and Cajal, but rather that all the fibers divided into a cephalic and caudal branch, nor was he able to find collaterals given off from the fiber before its division into these branches, although they were found constant enough on the branches, ending in three or more branchlets which terminated freely between the round cells forming the cinerea. In the spinal ganglia he has noted cells with two processes (bipolar), the peripheral process being thicker than the central. The neuroglia is composed of cells extending from the myelocœle to the periphery. Those immediately bounding the cavity were of brownish color while ectal to these were some more darkly colored and possessing two processes; the central process extends undivided to the cavity, while the peripheral one divides two or more times before it leaves the cinerea. Cells were also found without the central process. The glia fibers were followed to the periphery and were seen to end for the most part just ental to the pia with a greater or less enlargement, and were in no way connected with the pia. Spider cells as seen in the adult myel were not observed by Sclavunos in the larva.

As far as ordinary histological methods reveal its structure, the neuraxis of the *Desmognathus* might be very briefly characterized as consisting of an ental and an ectal layer: the ental or cellular layer of cinerea is composed of conical cells and varies in thickness in different regions, becoming quite attenuated at the roof of certain parts of the cavity. In the myel, the layer averages from six to eight cells deep, while in

the mesencephal, where the layer is thickest, the cells are from twelve to sixteen deep. The ectal layer or alba consists of fibers and general supporting substance; scattered around in this layer are isolated cells which appear to have drifted out from the entocinerea. These outlying cells are present in the myel as well as in the brain, but are especially numerous in the dorso-caudal and lateral regions of the cerebrum, where they undoubtedly represent an incipient cortex or ectocinerea.

Edinger (9), inquiring what sensations may be localized in this rudimentary cortex first appearing in the *Amphibia*, finds "that the cortex of these animals is connected by a strong system of fibers almost exclusively with the olfactory apparatus. The phylogenetically oldest cortex serves the olfactory sense, and has even thus early certain peculiarities which permit us to consider it closely related to the ammonshorn"; from which it is believed that the olfactory tract connects with higher centers at an earlier stage phylogenetically than any other nerve. Cajal, Van Gehuchten, Kölliker, and others have demonstrated that the first centers of the olfactory nerve lie in the pero or the most ectal layer of the olfactory bulb, and that the fibers connecting them with the cortex are properly projection fibers for the olfactory sense.

By means of the Golgi method, three forms of cells may be distinguished in the neuraxis of the *Desmognathus*: First, those next the neurocœle forming the ental boundary of cinerea, the endymal cells. They agree in all essentials with the descriptions of Oyarzun and others, being pyriform in outline, the blunt end of the cell bounding the cavity or occasionally protruding into it, and the peripheral end extending into a process which divides into numerous smaller ones in the cinerea as well as in the alba. The processes are irregular or ragged in outline and many of them extend as far as the periphery of the neuraxis, where they may end taperingly or by means of a slight enlargement. The processes are exceedingly dense and much interlaced, and without question form the greater amount of the supporting substance. The second form of cell is found at the ectal boundary of the cinerea and their outlines vary from a somewhat blunt pear-

shape to a distinct fusiform cell with the long axis extending perpendicularly to that of the endymal cells, or in other words more or less parallel with the periphery of the neuraxis. They are always characterized by the presence of more than one process. These processes were always found to extend parallel with or somewhat towards the periphery.

Between the endymal cells of the ental boundary and the dendritic cells of the ectal boundary of the cinerea, there exist intermediate forms which suggest very strongly that they, in the course of time, will develop into these ectal cells. The intermediate cells have only one process, which is of greater or less length according as the cell is near or far from the alba; this process very rarely divides before reaching the alba and these branches, although they may be traced some distance, are found to have a general trend toward the periphery; smaller branches are given off at intervals, and these may again subdivide. At the first bifurcation, the angle of divergence is very great, generally ninety degrees on even one hundred and eighty degrees; the other divisions are usually at acute angles, there being more or less of an enlargement at the point of bifurcation. The processes are comparatively smooth, the central end of the cell remains smooth and blunt and the neurite or axis-cylinder process does not spring from that end of the cell body. In various preparations, I have been able to detect a finer branch arising from one of the processes not far from the point of bifurcation or from the peripheral end of the cell itself, Figs. 53 *n*, 44 *n*, which I believe to be the neurite, because it could be traced a greater distance than the other processes; there were fewer branches given off from it and these were likewise of finer caliber than the neighboring dendrites and left the main stem at greater angles. In some instances the neurites appear so fine and delicate, that it quite naturally suggests the question that they, like the fibrin filaments of the amphibian blood, may have been overlooked by many because of their extreme delicacy.

The changes undergone by the cells of the spinal ganglia from the early "oppositipolar" condition to the unipolar have been well demonstrated by von Lenhossék and others. Fig. 52,

representing a section through the Gasserian ganglion of a larval lamprey, has been introduced in order to show the various transitional stages.

In the cells of the neuraxis of the *Desmognathus*, there is apparently an exact reversal of transition. Starting with the layer next adjacent to the endymal cells, we find the unipolar condition. Toward the ectal boundary of the cinerea, the cell process becomes very much shorter, until at the very margin of the cinerea the bifurcation of this process occurs at the cell itself, Figs. 44 and 49. The cells lose their pear-shaped appearance as if from ento-ectal pressure and assume a fusiform outline with a branching process extending from each end, Figs. 56, 58, 60. In other instances, there has been the appearance of a pyriform cell with one of its broad sides facing the alba, from which one or more processes grow out, Figs. 56, 58.

The conditions are so suggestive of the embryology of the neuraxis of higher animals as described by His, that a brief recapitulation may be permitted. The germ cells which give rise to the neuroblasts lie near the cavity between the spongioblasts or supporting structures. The protoplasm of the neuroblasts arranges itself on the side of the nucleus, away from the cavity, and this extending toward the surface becomes in the course of growth, the nerve fiber. The neuroblasts themselves migrate toward the periphery to form the mantle layer or cinerea; the fibers lying between the cinerea and the ectal surface represent the alba.

An interesting point arises in connection with the cells lying intermediate between the endyma and alba. According to Roller's idea (44) they might be regarded as nerve cells developing from endymal cells. Embryological evidence, as shown by His, seems to refute this method of evolution. The location of these intermediate cells would indicate that they were true nerve cells undergoing a change of form, in case of their migration toward the periphery; or, if the migration has occurred before the cell has developed its process, its form may be influenced by the direction of the growth of the processes, — these may follow the lines of least resistance. The

intermediate cells always send their processes toward the periphery; the fusiform cells send their main processes between the layer of cells and the alba, but their ends and their branches point toward the periphery. If the lines of least resistance were always followed by these processes it would seem as if they would, in the case of these more ectal cells, grow toward the cavity through the loosely arranged cells rather than through the dense interlacing of the fibers that comprise the alba. A plausible explanation for this fact seems to be that of polarization; that there is an inherent power in the cell itself controlling the direction of the growth of its process.

In the unipolar type of cell found in *Desmognathus* it may seem of doubtful propriety to differentiate one of the branches of this single process as a neurite, simply because it is longer than the others. The dendrites are in reality nothing more than prolongations of the cell body, and in the cortex of some higher forms the neurites are said to arise from them. Where these two appurtenances of the cell are well developed, it is quite probable that the dendrites subserve the important function of collecting the nerve impulse in transit, by means of induction through propinquity and the neurite of discharging it; in other words, the dendrites are cellipetal in function and the neurites cellifugal.

Does the single process arising from the intermediate cell in the *Desmognathus* represent a neurite breaking up into branches, — a somewhat advanced neuroblast, or does the process combine both neurite and dendrite, forming a neurodendrite? In the unipolar cells of the spinal ganglia of higher forms a portion of this single process is evidently cellipetal and another portion cellifugal, the nerve impulse passing from the peripheral fiber to the nerve cell and back through the common process of the cell to the central fiber. The evidences of fatigue shown by the cell after electrical stimulation of the peripheral fiber, in the experiments of Hodge, seem to confirm this view of the matter.

The unipolar cells in the central nervous system of *Desmognathus* are not strictly comparable to the unipolar cells of the

spinal ganglia, because the former do not show peripheral and central fibers provided with myelin, neurilemma, etc. If we cannot admit that a portion of this single process of the cell in *Desmognathus* is dendritic or cellipetal in function, then it must follow that the impulse must pass out from its cell of origin toward the periphery, but cannot again take a cellipetal direction in one of these unipolar cells. In these cells the conduction would seem to be cellifugal, and a physiological difference may be distinguished between these and the fusiform cells, for the latter have numerous processes, and conduction is probably both cellipetal and cellifugal, though it is worth noting that these processes extend for the most part toward the periphery.

The third type of cell of the *Desmognathus* was found only in the oblongata (Figs. 40 and 45); they are larger than the others, and in outline resemble the so-called multipolar cells.

The various forms undergone by the cells just described, confirmed to a greater or less extent by ontogeny, seem to indicate that the unipolar is the primitive type of the nerve cell in the central nervous system, and that it merges gradually into the fusiform stage; while the pyramidal cell — the highest and most typical form of a nerve cell — “may be imagined as evolved from a fusiform cell.”

Aside from the endyma, nothing comparable to neuroglia cells was noted except in an unsuccessful sublimate preparation where no satisfactory conclusion could be arrived at as to whether the appearance was really a cell or an irregular precipitate. The neuroglia cells described by Sclavunos in the *Salamandra maculata* and *Siredon* as having a central process as well as peripheral ones, were not demonstrable in the *Desmognathus*. I have, however, found such an appearance in the prosencephal of a larval *Petromyzon*. The cells were not far distant from the ental boundary, and in some cases the central process appeared to project even into the cavity.

In the myel, many preparations seemed to show a distinct difference of caliber between the fibers of the dorsal and ventral roots, the former being the coarser. In their passage from the periphery to the myel, the dorsal root fibers assume

very complex relations among themselves and with the ventral root fibers in the ganglion. There are three distinct nerve trunks given off from the ganglion (Fig. 62). The first and largest passes off in a ventro-lateral direction, and corresponds to the ordinary spinal nerve trunk as found in the majority of vertebrates; the second passes in a latero-caudal and somewhat dorsal direction, while the third extends dorsally. The second and third trunks are about the same size. Ventral and dorsal root fibers were found in all three (Figs. 54, 57, and 62). In the latero-caudal trunk, the dorsal root fibers bend upon themselves at quite an acute angle to enter the myel through the dorsal root; and in the dorsal trunk, where these fibers would have to bend at a very acute angle to enter the myel through this root, the bending could still be demonstrated. The ventral root divides near the myel, and sends fibers to both the latero-caudal and dorsal trunks, as well as to the ventral trunk.

The division of these fibers in the ganglion itself to form nerve trunks is an unusual occurrence, although their division just outside of the ganglion is not so infrequent. Schaffer (49) notes and figures a ganglionic division of the ventral and dorsal roots in the *Anguis fragilis*, and that in this crossing of the fibers there result two bundles, the larger of which, the *ramus communicans ventralis*, contains the motor elements of the dorsal root; the more slender bundle, the *ramus communicans dorsalis*, contains the sensory elements of the ventral root; so that there pass out from the ganglion two nerves. His, in 1888, showed that there enter into certain of the cranial nerves fibers which are not ganglionic in their origin, but, from their source, are evidently efferent, or motor, in function, and that in this apparently dorsal root there are found two elements, one of which, the efferent, Minot (30) calls the lateral root. This condition is also said to exist in some of the more cephalic of the cervical nerves; and Minot prognosticates that they may also be found in the spinal nerves as well. Van Gehuchten (14) a few months before the appearance of Minot's paper, had described the presence of efferent, or motor, fibers in the dorsal roots of an embryo chick, confirming what had been stated by

Cajal and Lenhossék. So that on this basis there may be recognized three real spinal nerve roots — dorsal, lateral, and ventral, the lateral being fused with the dorsal. The extension of the cinerea in some regions of the myel to form the so-called lateral horn may be correlated with the lateral root, and would tend to confirm the view of its identity. This precocious division of the fibers in the ganglia of the *Desmognathus* into three nerve trunks suggests quite naturally a possible correlation with the presence of the three nerve roots. In this animal the ventral root divides quite close to the myel, and some of the fibers bend quite abruptly to enter the dorsal and latero-caudal nerve trunks. I am rather disinclined, in this instance, to accept Schaffer's view that these connecting rami contain the motor elements of the dorsal root and the sensory elements of the ventral root, but believe that the fibers passing from these three nerve trunks into the dorsal root are of an afferent or sensory character, while those coming from the ventral root are efferent, or motor, in nature; because it seems entirely unnecessary in the dorsal and latero-caudal trunks, where both kinds of fibers are represented, as well as in the ventral, to reverse their course and send some of the motor elements through the dorsal root and some of the sensory through the ventral; or, to put it in another way: that the motor fibers from the dorsal root and the sensory (?) from the ventral arrange themselves in the ganglion in such a way that the nerves leaving the ganglion contain motor or sensory fibers only as the case may be. It is only just to say that Schaffer's paper deals almost entirely with the arrangement of the fibers in the myel itself, and that the arrangement in the ganglion is apparently an incidental observation; but his statement, if carried to its logical conclusion, amounts to what has just been said.

The fibers entering the myel through the dorsal root were seen in some cases to divide into a cephalic and caudal branch, while in other cases no division was noted. Collaterals are given off at various intervals into the cinerea; they end freely between the cells; the ends are somewhat swollen, and the whole collateral is relatively coarse in texture. The ganglion

cells, in the larvæ at least, are oppositipolar, and there is no apparent difference between the central and peripheral fibers with regard to caliber. In one instance (Fig. 37) there is shown a very distinct T-fiber, indicating a connection with an unipolar cell, but as no impregnation occurred in the cell an absolute connection could not be established.

In the Gasserian ganglion of a larval *Petromyzon* (Fig. 52) a unipolar cell is shown which has one central and two peripheral fibers. In a larval *Desmognathus* about two centimeters long there was seen a branch given off from the peripheral fiber near the cell, which entered the myel, passing in a caudal direction, and gave off at least one collateral. While this peripheral branch and its connection could be demonstrated with the utmost clearness shortly after the preparation was made, some three months afterwards enough fading had set in to totally obscure this connection. In those cases where the fiber divided into a cephalic and caudal branch no collaterals were seen to arise from the fiber before this division, and when the fibers remain undivided, they were always seen to pass toward the brain, giving off collaterals occasionally.

FIBER TRACTS.

In the *Desmognathus* the fibers of the prosencephalic and diencephalic commissures are chiefly of the amyelinic variety as determined by the Weigert method; as the fibers did not take the hematoxylin in any instance, while in the succeeding segments they stood out with a bright blue color, it seems reasonable to conclude that the absence of the color was due to the absence of the myelin of the fiber. The study of the amyelinic tracts was based upon Golgi preparations and the course and relations of the fibers in most cases were clearly made out. Under the amyelinic tracts are embraced the pre-commissure, callosum, supracommissure, and Meynert's bundle.

The *precommissure* is divisible into two parts, the *pars olfactoria* and the *pars temporalis*, Fig. 6. The former is the larger bundle and the fibers pass toward the olfactory lobes. The fibers of the *pars temporalis* curve around toward the

caudal end of the hemicerebrums. This commissure as seen in a medisection is quite high, projecting some little distance into the cavity from the apparent floor, Figs. 3, 13. The basal portion is partially made up by fibers from the basal prosencephalic tracts.

The *callosum* is separated from the dorsal portion of the precommissure by a single layer of cells. The fibers of the callosum arch dorsad like the letter U, Fig. 28, to help form the roof of the paracœles. They also bend cephalad in the roof, Figs. 12, 13, and may extend in a latero-caudal direction, while some, as has been noted by Osborn (38), seem to enter the diencephal or to mingle with the fibers of the supra-commissure.

The *supracommissure* is well developed in the *Desmognathus* and is in close connection with the habenæ. It takes a ventrocephalic course, sending some of its fibers into the callosal eminences or mesal walls of the hemicerebrums. A smaller bundle remains in the diencephal, while a still smaller proportion of the fibers arch around into the latero-caudal ends of the cerebrum, Fig. 5. Osborn and Bellonci consider that both commissural and decussation fibers are represented in this commissure, while Herrick believes that two distinct commissures should be recognized, instead of one dividing into two branches.

Meynert's bundle (*fasciculus retroflexus*, Edinger). This is a small but distinct bundle arising at the habenæ. It takes a latero-caudo-ventral direction and approaches the meson in the floor of the mesencephal in the region of the interpeduncular nidus. Osborn has found fibers running beyond this point in *Amphibia*, while Ahlborn states that he has traced them into the metencephal in *Petromyzon*.

The *postcommissure* is composed of myelinic fibers. It lies caudad of the epiphysis and at a little distance from the surface. Beginning at the postcommissure the fibers of the *commissura tecti optici* or sylvian commissure extend along the roof of the mesencephal as a thin transverse layer. The course of the postcommissure is more directly ventral than Meynert's bundle; its fibers soon become diffuse and I was unable to establish

their relation. According to Köppen (22) they end in the gray substance dorsad of the *pars peduncularis*. Osborn (38) finds a relationship between the oculo-motor nidus or nucleus and the "pale ganglion cells behind this nucleus."

The fibers of the *optic chiasma* sink into the base of the brain, a part of them crossing each other, and at a certain height associating themselves, as has been remarked by Köppen, into what is called Haller's transverse commissure. The other, the cephalic bundle of fibers, passes latero-caudad along the thalami and gemina expanding as they go and become associated with the tectum. Osborn (38) finds a tract of optic fibers coming from the prosencephal in both the *Urodela* and *Anura*, but states that it requires confirmation.

The *cerebellum* is composed largely of myelinic fibers arranged in two bundles of which the cephalic one lies also more dorsal and sends its fibers latero-cephalad into the gemina. They are perhaps comparable to a rudimentary valvula, Figs. 5, 15, 16. The caudal bundle extends toward the lateral portions of the oblongata and, as has been observed by Osborn and Köppen in other *Amphibia*, bear an intimate relation to the auditory and trigeminus nerves.

The fibers of a well-marked bundle lying in the diencephal and prosencephal are of both the myelinic and amyelinic variety in the *Desmognathus*. Various names have been applied to this tract. Herrick, in some forms that he has studied, has found it divided and names the parts the dorsal and ventral peduncles. Edinger calls it the basale Vorderhirnbündel; Osborn, the basal prosencephalic tract; Köppen, the rundes Bündel; Schulgin, the Bahn P. Analogon der Pyramiden. This tract becomes a distinct bundle of fibers in the region of the mesencephal and is situated nearer the ventral portion of the brain. The fibers remain quite compact through the thalami but radiate considerably in the prosencephal. Some of them, as has already been noted, bend mesad to help form the basal portion of the precommissure. Others turn toward the latero-caudal end of the hemicerebrums, while the most of them continue toward the olfactory lobes, but expand in the thickened part of the lateral cerebral walls, — the homologue

of the striatum. Toward the cephalic end of the diencephal there is a good-sized dorsal branch passing up dorso-caudad toward the tectum (Fig. 11).

The *posterior longitudinal fasciculus* (hintere Langsbündel) begins as a well marked bundle in the region of the corpus interpedunculare, and passes through the oblongata into the myel. It is composed of myelinic fibers, and through the oblongata there are many decussational fibers in close relation to these fasciculi, which suggest a comparison to the pyramid tracts of higher forms. In the *Desmognathus* there is no sharp line of demarcation between the peduncular tracts and the posterior fasciculi further than a less compact arrangement of the fibers at the point where the two systems would be supposed to meet.

In the *myel* the ventral and lateral columns are not very clearly differentiated from each other. Köppen finds that in the frog the ventral possess larger fibers than do the lateral columns. In the larva of *Desmognathus* the fibers of the dorsal columns are easily differentiated from the lateral in Golgi preparations on account of their greater coarseness. In the adult this distinction is not so apparent by ordinary methods. The ventral commissure lies just ventral to the myelocœle; the dorsal commissure is represented by a few transverse fibers which cut off dorsally some of the cells from the central mass. Köppen says that the dorsal commissure is not present in the frog; but that there are fibers going from side to side, only not joined into a distinct commissure. The *Desmognathus* shows occasional fibers of large caliber scattered through the ventral columns, as has been noted by Köppen in the frog. These are exclusive of the Mauthner's fibers, which are very distinct in the larva of *Desmognathus* and have also been noted in mature specimens, but less constantly. They are situated in the ventral columns, one on each side, at a little distance from the ventral fissure. Time has not permitted the unraveling of numerous other interesting and complex fiber arrangements in this important region.

Osborn (38) states that Mauthner's fibers are wanting in most of the *Urodela*, but records that Stieda had observed them

in the *Axolotl* (*Siredon*). Burckhardt (2) states that these fibers remain only in the larvæ of *Amphibia*. They have been investigated in the *Teleosts* by Mayser, and in the *Petromyzon* by Ahlborn, who distinguishes them from Müllerian fibers by their greater size. They have also been found later by Burckhardt (3) in the *Protopterus*, where they are of large size and exhibit a fibrous structure, which in horizontal sections is seen to be due to the entrance of fibers from without.

Sanders (47) also finds them well developed in the *Ceratodus*, but calls them multiaxial fibers. He investigates them at some length and the following is quoted from him :

“The peculiarity of their structure consists in the fact that from forty to fifty axis cylinders are contained in a single medullary sheath which is common to all of them. This sheath, notwithstanding its immense extent and thickness, has the appearance and structure of the medullary sheath of ordinary nerve fibers. The axis cylinders also resemble those found in other parts of the cord.

“Fulliquet, who appears to have been the first to investigate microscopically the brain of *Protopterus*, describes two large fibers occupying a corresponding position in the spinal cord of that animal ; these he terms Mauthner's fibers, but from his plates and the terms in which he mentions them, I should imagine that he refers to Müllerian fibers. The distinction is essential, for while the latter are unprovided with medullary sheaths, the former have them unusually well developed.

“The multiaxial fibers commence at the posterior end of the spinal cord, and are first met with opposite the hinder end of the abdomen ; here they consist of a very few axis-cylinders enclosed in a comparatively small medullary sheath ; the axis-cylinders gradually increase in numbers as the fibers proceed forward, but the increase is not uniform ; at about the middle of their course they become much smaller, and after a few sections again enlarge to the original diameter. But this diminution does not occur at the corresponding point on the opposite side ; but the fiber of one side first suffers a diminution, then that of the other side.

“Occasionally axis-cylinders may be seen escaping singly or

in groups, passing through the medullary sheath without diminution of size into the field of the ventral columns. This fact accounts for the diminution in size at various points in the course of the fibers.

"The shape of the multiaxial fibers varies according to the part of the spinal cord in which they are observed; at the posterior end they are elliptical, towards the center they are round, and further forward they are almond-shaped.

"At a short distance posterior to the point where the facial emerges from the brain, this axis-cylinder is the only one remaining, all the others having disappeared, the whole fiber having in the meantime diminished in size. The fiber has now quite the appearance of the Mauthner's fiber of the Teleostei, consisting as it does at this point of a single large axis-cylinder surrounded by a thick medullary sheath. Immediately in front of this spot the axis-cylinder of one side decussates with that of the other, the place corresponding with the position of the decussation of the Mauthner's fibers in the Teleostei."

SUMMARY.

1. The preservation of so many embryological features both in the morphology and histology of the neuraxis of the *Desmognathus* is quite remarkable. Throughout the whole length of the tube there may be recognized the homology of the three layers as described by His. In the early embryo the ental layer is composed of epithelium or endyma; the middle or mantle layer consists of the cinerea, and the ectal layer of alba. The nerve cells appear to be scarcely more than fairly well developed neuroblasts. The regeneration of lost parts in the Urodeles is well known, and this phenomenon may be more or less dependent upon the embryonic condition of the elements of the neuraxis and their power of rapid growth utilized in this direction.
2. The cephalo-caudal compression of the brain parts is quite marked and undoubtedly exerts a strong influence upon the peculiar relations of the seventh and eighth nerves.
3. The crista in the *Desmognathus* is nothing more than a membranous intrusion of pia into the aula.

4. The community of origin of the precommissure, callosum, and hippocampal commissure or fornix is shown and a slight differentiation of the latter in the *Desmognathus* is suggested.

5. The existence of a *ligamentum dentatum* has been demonstrated in the adult and larval forms. In the latter, where the myel but partially fills the spinal canal, the ligament assumes a cord-like appearance with a peripheral and central attachment, and supports the myel in its proper position. In the adult there is no peripheral attachment, but in transections it appears as a thickened pad against the wall of the myel between the dorsal and ventral roots.

6. The evidence as to the intermediate forms of the nerve cells lying between the endyma and the peripheral boundary of the cinerea, and their transition from the pyriform or unipolar to the fusiform or multipolar condition, and the apparent reversal of transition of the cells as compared with those of the spinal ganglia.

7. The dichotomous arrangement of the dendritic branches.

8. The identification in this animal of myelinic and amyelinic fiber tracts by the Weigert and Golgi methods. The amyelinic being confined to the prosencephal and the most of the diencephal, and represented by the precommissure, callosum, supracommissure, Meynert's bundle, and a portion of the peduncular tracts.

9. The discovery in larval and adult *Desmognathus* of three distinct nerve trunks emerging from the spinal ganglia: the ventral, dorsal, and latero-caudal, each including dorsal root and ventral root fibers, and from the mixed nature of these fibers in each trunk, the function of all three is doubtless the same.

10. The Mauthner fibers were demonstrable in an adult specimen of the *Desmognathus* but less easily than in the larvæ.

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DESCRIPTION OF FIGURES.

With the exception of Figs. 18 and 48 (which were outlined from photographs) and Figs. 20, 21, 22, 23, and 24 (which were traced from other figures), all were outlined by the aid of Abbe's *camera lucida*, and some of the details filled in free hand. All of the figures of transections are arranged to face the observer, so that their right sides correspond to his left. In the case of the frontal and transections, the outlines of the figures were drawn from one series of sections, and the fiber tracts drawn in from another series — amyelinic from Golgi preparations, and the myelinic from Weigert's.

REFERENCE LETTERS.

| | | | |
|---------------|--------------------------|-------------------|------------------------------------|
| <i>a.</i> | aula. | <i>Mesen.</i> | Mesencephal. |
| <i>alb.</i> | alba. | <i>Meten.</i> | Metencephal. |
| <i>aplx.</i> | auliplexus. | <i>Mf.</i> | Mauthner's fibers. |
| <i>bv.</i> | blood-vessel. | <i>mle.</i> | myelocœle. |
| <i>cal.</i> | callosum. | <i>mtc.</i> | metacœle. |
| <i>cb.</i> | cerebrum. | <i>mtplx.</i> | metaplexus. |
| <i>cbl.</i> | cerebellum. | <i>n.</i> | neurite. |
| <i>cblf.</i> | cerebellar fibers. | <i>nch.</i> | notochord. |
| <i>ce.</i> | callosal eminence. | <i>obl.</i> | oblongata. |
| <i>ch.</i> | chiasma. | <i>of.</i> | optic fibers. |
| <i>ci.</i> | corpus interpedunculare. | <i>olf.</i> | olfactory. |
| <i>cin.</i> | cinerea. | <i>on.</i> | optic nerve. |
| <i>coll.</i> | collateral. | <i>or.</i> | preoptic recess. |
| <i>cr.</i> | crista. | <i>p.</i> | porta. |
| <i>cto.</i> | commissura tecti optici | <i>paraph.</i> | paraphysis. |
| <i>d.</i> | dendrite. | <i>pars olf.</i> | pars olfactoria. |
| <i>dc.</i> | diacœle. | <i>pars temp.</i> | pars temporalis. |
| <i>Dien.</i> | Diencephal. | <i>pc.</i> | paracœle. |
| <i>dplx.</i> | diaplexus. | <i>pcs.</i> | postcommissure. |
| <i>dr.</i> | dorsal root. | <i>pdle.</i> | peduncular tract. |
| <i>dtr.</i> | dorsal trunk. | <i>plf.</i> | posterior longitudinal fasciculus. |
| <i>ec.</i> | epicœle. | <i>pplx.</i> | paraplexus. |
| <i>Epen.</i> | Epencephal. | <i>pres.</i> | precommissure. |
| <i>epiph.</i> | epiphysis. | <i>Prosen.</i> | Prosencephal. |
| <i>gln.</i> | ganglion. | <i>prc.</i> | prosocœle. |
| <i>gm.</i> | geminum. | <i>rc.</i> | rhinocœle. |
| <i>hb.</i> | habena. | <i>Rhinen.</i> | Rhinencephal. |
| <i>hyph.</i> | hypophysis. | <i>sps.</i> | supracommissure. |
| <i>infid.</i> | infundibulum. | <i>splx.</i> | supraplexus. |
| <i>lctr.</i> | latero-caudal trunk. | <i>t.</i> | terma. |
| <i>ld.</i> | ligamentum dentatum. | <i>vr.</i> | ventral root. |
| <i>Mb.</i> | Meynert's bundle. | <i>vtr.</i> | ventral trunk. |
| <i>mc.</i> | mesocœle. | | |

EXPLANATION OF PLATE XIII.

FIG. 1. The brain of *Desmognathus fusca* from the ventral aspect. $\times 20$.

FIG. 2. The same from the dorsal aspect. $\times 20$.

FIG. 3. A mesal section of the brain, reconstructed from sagittal sections.
 $\times 20$.

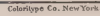
FIG. 4. Sinistral aspect. $\times 20$.

FIGS. 5-7. Frontal (horizontal) sections at different levels. The blue lines represent the myelinic nerve-fibers, as shown by Weigert's hematoxylin stain. The dark lines represent the amyelinic fiber-tracts. $\times 20$.

FIG. 8. A frontal section at the base of the brain, showing the arrangement of the fibers at the chiasma, and the preoptic recess just cephalad; also the thickening of the caudal wall of the infundibulum where the hypophysis lies. $\times 20$.

FIG. 9. The most dorsal of the frontal sections, showing the origin of Meynert's bundle and a portion of the postcommissure. $\times 20$.

FIG. 10. A section at a little deeper level. It shows the relation of the paraphysis to the supraplexus, and of Meynert's bundle to the postcommissure.
 $\times 20$.



EXPLANATION OF PLATE XIV.

FIG. 11. A sagittal section, showing the division of the peduncle into a dorsal and ventral branch in the diencephal. The relation of Meynert's bundle and the postcommissure are well shown. $\times 20$.

FIG. 12. Shows the relations of the callosal fibers to those of the supracommissure, and the apparent separation of the rhinocœle from the paracœle by the lateral bulging of the callosal eminence. $\times 20$.

FIG. 13. Shows the thickening of the caudal wall of the infundibulum and the preoptic recess. $\times 20$.

FIG. 14. A section near the median line, showing the relation of the plexuses to the cavities. $\times 20$.

FIGS. 15-16. Show the direction of the two fiber tracts of the cerebellum.

Fig. 15 is more dorsal, and shows a single bundle. $\times 20$.

FIG. 17. A transection through the rhinencephal at the attachment of the olfactory nerves. $\times 40$.

FIG. 18. A transection through the body of a 17-mm. larva to show the incomplete filling up of the spinal canal by the myel, and the relation of the *ligamentum dentatum* to the dorsal and ventral roots, and its peripheral attachment.

FIG. 19. Transection through the myel. $\times 190$.

FIGS. 20-24. These figures are introduced to represent a series illustrating the changes undergone by the terma and its commissures, phylogenetically, with a view to strengthening their homology.

Fig. 20 is the mesal region of these parts in *Desmognathus*;

Fig. 21, of *Cryptobranchus*;

Fig. 22, of *Rana*;

Fig. 23, of *Emys*;

Fig. 24, of *Anas* (duck).

(Figs. 21-24 are from Osborn.)

FIG. 25. Transection through the rhinencephal. $\times 40$.

FIG. 26. Transection through the prosencephal, with the cephalic end of the supraplexus just appearing. $\times 40$.

FIG. 27. Shows the supraplexus dipping down between the two hemicerebrums, and extending laterally through the portas to form the paraplexus. $\times 40$.

FIG. 28. Shows the relation of the callosum and precommissure to the adjacent parts. $\times 40$.

FIG. 29. A transection through the diencephal, with the caudal end of a hemicerebrum on each side. $\times 40$.

FIG. 30. Transection through the mesencephal, with the infundibulum. $\times 40$.

FIG. 31. Same, farther caudad. $\times 40$.

FIG. 32. Mesencephal, showing the separation of the cinerea of the roof into two layers. $\times 40$.

FIG. 33. Section at about the level of the seventh and eighth nerves. $\times 40$.

FIG. 34. Caudal end of oblongata, near the myel. $\times 40$.

FIG. 35. Shows the cerebellum, with the overlapping geminum. $\times 40$.

FIG. 36. Transection through the metencephal. $\times 40$.

EXPLANATION OF PLATE XV.

FIG. 37. Transection through the myel and spinal ganglion to show the fibers and the passage of the dorsal root-fibers into the myel. $\times 190$.

FIG. 38. Shows the precocious division of the ventral root. From near the middle of the length of the body. $\times 190$.

FIG. 39. Cell from the roof of the mesencephal. $\times 190$.

FIG. 40. Multipolar cell from the oblongata. $\times 190$.

FIG. 41. Cell from the occipital region of the cerebrum. $\times 190$.

FIG. 42. Cell from the mesencephal. Nerve cells, with their processes mostly branching at the alba. $\times 190$.

FIG. 43. Transection of myel; tail region. $\times 190$.

FIG. 44. Transition cells from the mesencephal. $\times 190$.

FIG. 45. Multipolar cell from the oblongata. $\times 190$.

FIG. 46. Transection of the cerebellum, to show the beaded or varicose condition of the fibers. $\times 190$.

FIG. 47. Cell from the dorsal portion of the myel, with its neurite crossing to the opposite side; near the vent. $\times 190$.

FIG. 48. Transection of myel, to show a transitional form of cell located at the margin of the entocinerea; just caudad of the arms.

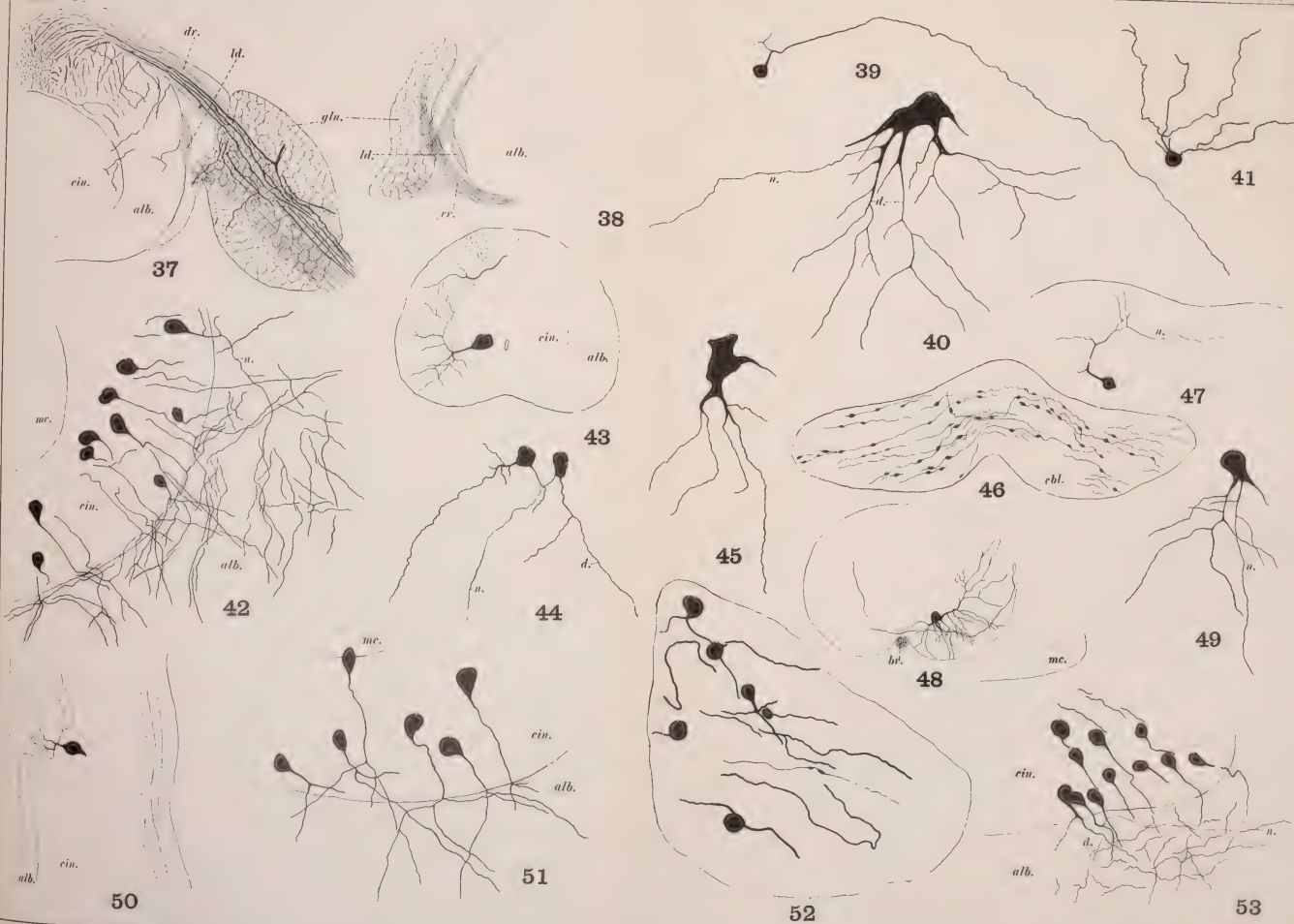
FIG. 49. A transition cell. $\times 190$.

FIG. 50. Frontal (horizontal) section of myel. $\times 190$.

FIG. 51. Region of mesencephal, to show the division of the cell-processes at the alba, and occasionally in the cinerea. $\times 190$.

FIG. 52. Transection through the Gasserian ganglion of the *Petromyzon*, showing transitional stages of the ganglion cells; one of the cells is connected with three processes. $\times 190$.

FIG. 53. From the mesencephal, similar to Figs. 42 and 51. $\times 190$.



EXPLANATION OF PLATE XVI.

FIG. 54. Transection of myel, the dorsal columns being characterized by the cut ends of coarser fibers. There are also shown a few fibers of the dorsal commissure cutting off some of the cells dorsally from the main portion. The ganglion, with its two roots and three nerve trunks, is, likewise, shown. Near the arms. $\times 65$.

FIG. 55. Transection of myel, showing the course of the cell processes to form the ventral commissure. $\times 65$.

FIG. 56. To show the appearance of the cells at the margin of the entocinerea; vent region. $\times 65$.

FIG. 57. To show the arrangement of the spinal nerve-roots and trunks; from the region of the stomach. $\times 65$.

FIG. 58. Fusiform cell; from the myel, toward the tail. $\times 190$.

FIG. 59. Transition cell; vent region. $\times 190$.

FIG. 60. Fusiform cell; vent region. $\times 190$.

FIG. 61. Fibers from the dorsal column, with their collaterals. $\times 190$.

FIG. 62. A figure reconstructed from Figs. 38, 54, and 57.

FIG. 63. Shows the fibers from the dorsal root, some remaining undivided, and others dividing into a cephalic and caudal branch from which are given off collaterals. A few spinal ganglion-cells are also shown. $\times 190$.

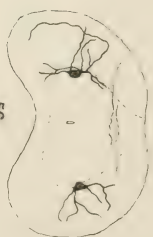
FIG. 64. Cell from the olfactory region. $\times 190$.



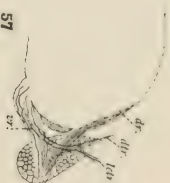
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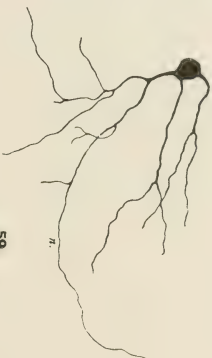
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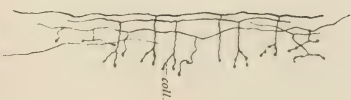
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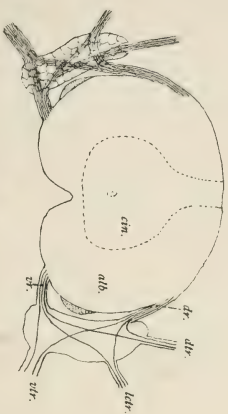
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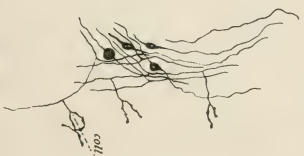
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64

THE SENSORY CLUBS OR CORDYLI OF LAODICE.

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By the invitation of Marshall McDonald, U. S. Commissioner of Fisheries, I was enabled to spend the summer of 1888 at the laboratory of the Commission at Woods Holl.

I found a species of *Laodice* there in abundance, and as the Hertwigs have been led by theoretical considerations to believe that the incipient stages in the evolution of the velar, ectodermal, marginal vesicles of the campanularian medusae (*Vesiculatae*, *Hertwig*) are to be sought for in this genus and its allies, I improved the opportunity to study its bell-margin and velum.

Other employment has prevented me from preparing my results for publication until now, although they bear upon several general questions.

I did not find incipient stages in the history of ectodermal sense-vesicles, but I did find evidence that the marginal clubs of *Laodice* are endodermal sense organs, agreeing in all essentials with the so-called "auditory clubs" of those medusae which Haeckel includes in the sublegion *Trachylinae*; the *Narcomedusae* and *Trachomedusae*. In the marginal clubs of *Laodice* we have these sense organs in a very primitive and simple condition, which corresponds to the incipient stages in their ontogeny in the *Trachylinae*.

This fact does not indicate that the *Trachylinae* are descended from a campanularian medusa like *Laodice*, but it does show that the distinction between the hydroid medusae and the *Trachylinae* is by no means so profound and fundamental as the books would lead us to believe.

In their account of the marginal vesicles, "*Hörbläschen*" and "*Hörgruben*," of the campanularian medusae, "*Vesiculatae*," the Hertwigs show that these organs are to be found in several

grades of perfection, and that a series of intermediate forms connects the simple open velar pouch of *Mitrocoma* with the closed vesicle of *Aequorea*.

Inasmuch as these organs are restricted to the higher members of the group, "*Vesiculatae*," and here form a progressive series, and as the group is a homogeneous and natural one, they hold it probable, p. 154, that the ectodermal marginal sense organs are not actually absent in the lower forms, but that they are probably present in a condition so simple and primitive that they have been overlooked.

A very similar opinion was advanced in 1857 by McCrady who suggests "the probability from the analogy of *Thaumantias* and *Tiaropsis* with *Eucope*, that these ocellate species will be found characterized by such marginal capsules" during their younger stages.

The Hertwigs say, however, that this is not the only alternative, for it is possible, although they do not think it probable, that these simple and primitive medusae may, at the present day, be in the condition which prevailed before the first trace of marginal vesicle was acquired.

My studies of *Laodice* show that neither of these alternatives holds true, but that we have, in its well-known marginal clubs, sense organs of a type which, according to the books, never occurs in campanularian medusae.

Laodice is neither without marginal sense organs nor does it present incipient stages in the evolution of ectodermal sense vesicles.

It has sense organs of very simple and primitive structure but they are not velar, nor ectodermal, nor are they connected with the lower nerve ring. They are endodermal, they spring from a nerve ring above the velum, and they are identical in all their anatomical relations with the sense-clubs of the *Narcomedusae* and *Trachomedusae*.

They are minute transparent clubs, attached by slender but rather stiff stalks to the bell-margin and they are well shown in Fig. 1. This is an oral view of a *Laodice* from the Bahama Islands. It has thirty-two large primary tentacles, each with a basal bulb and a large black ocellus ; alternating with these are

thirty-two solid accessory tentacles ; and in the radii of these there are thirty-two marginal clubs, and close to the base of each club there is a small ocellus, which is not on the club but on the marginal ring.

These marginal clubs are found in several species of ocellate campanularian medusae, and they have been noted and figured by A. Agassiz, and also by Haeckel who says that they are probably organs of special sense.

A. Agassiz gives a good figure of them in *Phytogena lactea* (*N. Amer. Acalephs*, p. 138, Fig. 224), but only says of them in this species that "between every two tentacles is found a club-shaped appendage, made up of large cells somewhat like those of *Lafoea calcarata*." His figure of them in this latter species, which is a *Laodice* according to Haeckel's system, is very obscure (Fig. 187). He says of them (p. 123): "We find still a third kind of tentacle ; club-shaped appendages made up of large polygonal cells, perfectly transparent, one or two sometimes placed between each of the larger tentacles."

Haeckel, who proposes for them the name *Cordyli*, says (*System der Medusen*, p. 118): "Marginal clubs, or *Cordyli*, are less frequent, on the whole, than *cirri*, and they may occur together with or without *cirri*. I have, so far, found these organs, which have been overlooked by most of the preceding authors, only in ocellate *Leptomedusae* (*Thaumantidae* and *Cannotidae*), while they are entirely absent in the vesiculate *Leptomedusae* (*Eucopidae* and *Aequoridae*). Among the *Thaumantidae* they occur in *Laodice*, *Melicertissa*, and *Melicertidium*, and among the *Cannotidae* in *Staurodiscus*, *Ptychogena*, *Berenice*, and *Toxorchis*. They are numerous and are usually distributed irregularly between the tentacles and *cirri*. The narrow stalk of the marginal club is always much smaller than the enlarged rounded tip, which, in some cases at least, if not in all, carries sensory cells with tactile bristles. They are not, therefore, to be confused with the tentacular buds, which have an enlarged base and a tapering tip. The *cordyli* are *probably organs of special sense*, possibly substitutes for the marginal vesicles, since they occur only in the *Ocellatae* and are not found in the *Vesiculatae*."

On p. 123, he says, "Among the more highly developed Thaumantidae there are associated with the tentacles numerous solid, oval, marginal clubs which are, in general, no longer than the tentacular bulbs. On the constricted stalk of the club there is often an ocellus."

Fig. 4 shows part of the bell-margin of a specimen of *Laodice* without accessory tentacles, from Woods Holl, magnified 360 diameters (Bauch & Lomb, $\frac{1}{8}$ objective. 2. eyepiece). The drawing is from an osmic acid specimen mounted in balsam. Two of the marginal clubs are shown on the right, alternating with the tentacles, and each placed upon the axial or velar side of a sensory pad or thickening of the upper nerve ring. Two tentacular bulbs are also shown, each with a large ocellus and a number of scattered pigment spots upon its axial or velar surface. Below these organs the vacuolated endoderm cells of the circular canal are shown, below the level of the transparent velum, which is not represented. On the left the circular canal, one of the tentacles, and the sensory pads of two of the marginal clubs are shown in optical section.

Fig. 5 is part of a vertical section through the bell-margin of a specimen from Woods Holl, of a *Laodice* which, like the one shown in Fig. 1, has an accessory tentacle in the radius of each marginal club. The section is from an osmic acid specimen, and the drawing is magnified 1380 diameters (Bauch & Lomb, $\frac{1}{12}$ homogeneous immersion, and 1. eyepiece).

The section passes through the base of one of the solid accessory tentacles, *a*; and through the axis of one of the marginal clubs, *b*. The space marked *c* is the gelatinous bell; *d* is the exumbrella; *e*, the subumbrella; *f*, the velum; *g*, the cavity of the circular canal; *h*, the place of the lower nerve ring, according to Hertwig; *i*, the upper nerve ring and sensory epithelium.

Fig. 6 is a section through the attached end or stalk of a club in a plane at right angles to Fig. 4, or horizontal.

These sections show that the sense organ consists, like that of the *Narcomedusae*, of two parts, a sensory pad formed by the thickening of the sensory epithelium of the nerve ring, and a free tentacle. The tentacle consists of a central axis of

modified endoderm cells, derived from the endoderm of the circular tube under the nerve ring; and a covering of ectodermal epithelium continuous with that of the nerve ring. My osmic acid specimens do not show sense hairs, either on the nerve ring or the club, although Haeckel assures us that in some cases at least, if not in all, the clubs carry tactile bristles.

Fig. 7 is a section through the base of one of the primary tentacles of the same specimen, and comparison with Fig. 5 will show that the sense club in the one section occupies very nearly the same position in relation to the velum and the upper nerve ring that the ocellus occupies in the other.

The stalk of the club is solid, but in the axis of the enlarged portion there is a remnant of a cavity, and Figs. 5 and 6 show that there is a deep pouch or diverticulum from the cavity of the circular tube, pushing down among the large vacuolated, endoderm cells nearly to the stalk of the club, which, undoubtedly, arises as a hollow tentacle.

There is only one club on the bell-margin of the young *Laodice* shown in Fig. 3, and in this the cavity reaches nearly to the base of the stalk.

The endodermal axis of the sensory club of the *Narcomedusae* is solid, and it is not directly continuous with the endoderm of the circular tube as it is in *Laodice*, for while the Hertwigs have shown that this continuity is found during the early stages in the development of the sense club, the connecting axis of endoderm cells disappears before the club acquires its perfect form.

The sense clubs which the Hertwigs have studied in the *Narcomedusae* and *Trachomedusae* differ greatly among themselves, although these authors show that they all may be reduced to a common plan.

Their account of these organs, which they regard as organs of hearing, may be briefly summarized as follows:

In the *Aeginidae* they are tentacle-like bodies attached to the bell-margin in connection with the upper nerve ring, and projecting directly into the surrounding water. They consist of two parts united by a slender stalk; a basal portion which may be regarded as a local enlargement of the nerve ring, and a

peripheral portion which is placed, like a small tentacle, on this enlargement. They call the first, the auditory pad, "Hörpolster," and the second, the auditory club, "Hörkolbchen."

Some of the Trachynemidae, *Aglaura*, for example, have similar organs, while in others the sensory pad is folded upwards around the club in such a way as to shut it in to a sensory vesicle, which may be open distally or completely closed.

In the Geryonidae the sensory vesicle which is thus formed is imbedded in the gelatine of the bell, and is thus shut off from all contact with the surrounding water.

Greatly as these various organs differ among themselves, the Hertwigs show that all are constructed on a common plan, and that they present three successive stages in the evolution of a peculiar sense organ which attains to its greatest complexity and perfection in the Geryonidae.

Among the forms which these authors studied *Cunina lativen- tris* has the organs in the simplest and most primitive condition. In this species the sensory pad is an insignificant thickening of the marginal nerve ring without any well marked boundary. It is wider tangentially than radially and forms a sort of pedestal, from the centre of which the sensory club arises. This is a cylindrical body like a rudimentary tentacle. Its basal end is much constricted to form a short stalk which joins it to the pad. Its free end is, on the contrary, somewhat enlarged, and it usually contains two concretions, the peripheral one largest. The sensory pad is nothing more than a thickening of the nerve-ring. The sensory club is made up of two sharply contrasted parts: a cylindrical axis, and a sensory epithelium with long, stiff hairs. The sensory epithelium is continuous at the base of the club with the epithelium of the nerve-ring, and it is separated by a supporting layer from the axial portion which consists of a single row of large flattened cells, like those in the axis of the tentacle of a hydroid. The early stages in the development of the club show that these axial cells are derived from the endoderm of the circular tube or its equivalent, although the fully formed club is not imbedded in the pad but attached to its surface, and the only trace of the

endoderm origin of its axis is a fibrous cord which penetrates the thickness of the pad and joins the supporting layer of the club to that of the bell-margin.

Careful study of the figures and descriptions of the marginal sense organs of the Narcomedusae and Trachomedusae which are given by the Hertwigs will show that while the sensory club of *Laodice* is much simpler than any of them, it is nevertheless essentially like them in structure and in all its anatomical relations, and that it corresponds to an incipient stage in their development before the endodermal axis loses its connection with the endoderm of the bell-margin and acquires concretions.

The cordyli of *Laodice* have long been known, and we have seen that Haeckel suggests that they may possibly be organs of some special sense. We have therefore to inquire why their agreement with the sensory clubs of the Trachylinae has escaped notice.

The most conspicuous feature in the structure of these clubs, in the Trachylinae, is the presence of solid concretions or crystals; they are universally regarded as hearing organs, and this interpretation derives most of its support from the presence of these heavy bodies or "otoliths." It is only natural for those who hold this opinion to assume that organs without "otoliths" cannot be ears, and that the cordyli of *Laodice* must therefore belong to a different category from the "auditory clubs and vesicles" of the Trachylinae.

Examination of the evidence that these are hearing organs is therefore in place. This opinion, which was not new when the memoir of the Hertwigs appeared in 1878, is now regarded as firmly established by their researches.

In the second part of their memoir they introduce the subject in the following words:—

"In our morphological description we have hitherto spoken of *hearing organs*, of *auditory cells*, and of *otoliths* without examining the ground for the use of these terms, and we shall now make good this omission by a comprehensive discussion of the physiological significance of the organs of which we have described the anatomy."

"We cannot here cite physiological experiments for, much as they are to be desired, none have yet been made either by us or by others. Our interpretation rests, therefore, on structural analogy alone, the value of which in estimating the nature of sense organs we have already set forth."

The pages which follow this extract show that the analogy upon which the authors rely is for the most part with sense organs which we have only the same reason for regarding as auditory organs; that is, although their structure seems to be adapted for hearing, there is little experimental evidence that they serve this purpose.

It is very probable that the sense vesicles of molluscs, crustacea, the brachiopods, doliolum, and many other invertebrates, and of many medusae may give sensations of sound, but it by no means follows that this is their only or their primary function.

Darwin and Dohrn have shown that many organs which now perform a definite specialized function were acquired for a different purpose, and afterwards came to perform, in a secondary, incidental way what at last became their chief function.

It is easy to understand that organs of vision may have been useful for vision from the first and may have been evolved directly for this purpose; that all the incipient stages in their history may have related to light, but it is very hard to believe that this is true of hearing organs.

All organisms which live in the light are exposed to its action for a considerable part of their lives, and its violent and rapid waves produce molecular and chemical changes in their structure. They do not act upon the organism as a mass, but they affect its most intimate structure. Their influence is in no sense psychological, for while they come from the outer world their action is identical with that of chemical changes which are set up within the body, although the first beginnings of sensation, no doubt, consisted of the production by natural selection of adaptive adjustments to constant external influences, as distinguished from those of internal origin.

As light is among the most persistent elements in the external environment, we have such phenomenon as heliotropism

or response to light in organisms which we cannot regard as sensitive to light. Susceptibility to the influence of light is not a property of the organism as a mass, but of the substance of its cells, and the first step in the evolution of a sense of vision is the specialization, in certain differentiated cells, of a property which inheres, to a lesser degree, in all the cells.

The aggregation of these cells in a special sense organ is only one more step in the process, and there is no difficulty in understanding that every one of the incipient stages in the evolution of eyes may have related to light.

The case of hearing is very different. There is no constant environment of sound as there is of light, nor does sound have any definite physiological influence apart from its action on sense organs. In these it does not act on the sensitive protoplasm, but it excites their tactile sensibility by variations in their contact with more massive bodies which are thrown into vibration by the waves of sound.

It is not probable that the incipient stages in the evolution of hearing organs were acquired for the purpose of hearing, and it is, to say the least, not impossible that the auditory organs of some animals may have arisen by the modification of sense organs which, while acquired for a different function, have proved, incidentally, to be sensitive to vibrations of sound.

• As the soft, watery, homogeneous medusae float in the ocean their specific gravity approaches more nearly to zero than that of any other animal with the power of voluntary motion, and their mobile, loaded, sense clubs and sense vesicles are well adapted for giving them the sensation of weight.

It is hard for us with our heavy bodies to appreciate the value of the sensation of weight or the necessity of special sense organs for perceiving weight, but a little reflection will show that we owe to it the difference between upwards and downwards and sideways; the perception of existence in space.

Gravity is the most constant of all the phenomena of the external universe, and the sensation of weight may possibly be the basis of all conscious life, the substratum which underlies all other sensations.

The sensation of weight comes to us in a thousand ways, by the aid of all our sense organs, and without the intervention of any of them. We never lose our consciousness of weight except in the delirium of nightmare, and it is so hard to imagine its absence that the perception of space is usually considered a necessary condition of mind.

While all animals are ponderable bodies, the sensation of weight is not due to weight but to specific gravity, to the difference between the weight of the body, or some part of the body, and that of its environment, and if the body be homogeneous, and its specific gravity approximate to zero, there can be little consciousness of weight, or perception of that difference between upwards and downwards and sideways, without which I find myself unable to conceive of voluntary motion or an external world.

Sight and hearing, no doubt, involve the conception of space, and no conscious animal which sees or hears can be without space-relations ; although we may lose sight, hearing, and even the tactile sense without losing our grasp on the external world. If with these the sense of weight were also lost the world around us would become

“ A great vacuity, a dark, illimitable ocean without bound,
Without dimensions; where length, breadth and height,
And time and place are lost.”

Now, while I do not assert that the medusae are conscious animals, they are beyond question endowed with the power of spontaneous motion, a power which could not be called into exercise unless movement brought about some change in external stimuli. If all the sensations are the same whether an animal moves or not, why should it move?

In the simple world of a jelly-fish the most important diversity is at the surface or the bottom and its movements are in some way adjusted to this relation.

Now if by any means top or bottom could be fixed all other dimensions of space would also be determined.

We can imagine two ways of fixing these points by special senses.

As the surface of the ocean is the chief source of light, a medusa with eyes may guide itself so as to approach or recede from or move parallel to the surface.

Ocelli too simple to form images may serve this purpose, which has no doubt been an important factor in the early history of visual organs, although eyes are not very well adapted for this use, whether they are simple or highly perfected, for it is difficult to believe that slight movements in the transparent water of the ocean can make any perceptible difference in the amount of light.

If the problem of giving a sense of direction to a disk-shaped or bell-shaped animal which always moves towards one pole of its central axis in a medium of its own density and which has its central nervous system placed around its edge, were to be solved, the simplest and most effective plan would be to attach movable weights, at regular intervals, to the nerve-ring, so that when one edge of the bell is raised and the other depressed the nerve-ring shall be differently affected on the upper and lower sides while movement upwards shall give one sensation and movement downwards a different one to the whole nerve-ring.

The so-called "auditory clubs" are, obviously, adapted for giving to the medusae this all-important sense of direction—a sense which they show, by every movement, that they possess.

Among the veiled medusae the sense clubs are found in three stages of perfection:

1. In the *Thaumantidae* and *Cannotidae* they are simple clubs, with an enlarged tip, united by a narrow stalk to a sensory eminence on the nerve ring;

2. In the *Narcomedusae* (Haeckel) and in the *Agauridae* among the *Trachomedusae* (Haeckel), the enlarged club-shaped tip of the projecting club is loaded with calcareous concretions;

3. In most of the *Trachynemidae* the sensory eminence is raised up around the club in such a way as to inclose it in a sensory vesicle, which is imbedded, in the *Geryonidae*, in the gelatine of the bell.

Comparison of the figures here given with those which the Hertwigs give will show that we here have the same organ in three successive stages of perfection. It is also clear that

each successive stage is adapted for increasing its efficiency as a *weight organ*, although it is only in the last and most perfected stage that it affords any basis for the analogy with the "auditory organs" of other animals upon which the Hertwigs base their belief that the sense clubs are ears.

There is no improbability in the view that the closed vesicles, with movable concretions, respond to sound vibrations, and function as auditory organs; but the view that they performed this function during their incipient stages presents many difficulties, which disappear as soon as we recognize the value of these incipient stages as means for giving to the floating medusa the sensation of space, which is a necessary condition for the perception of an external world and for conscious individuality.

THE SIGNIFICANCE OF THE MARGINAL SENSE-ORGANS IN THE SYSTEMATIC ZOÖLOGY OF THE VEILED MEDUSAE.

The view that the veiled medusae which are set free from hydroid cormi are distinguished from those with direct development by a fundamental difference in the structure of their marginal sense-organs was first put forward by the Hertwigs.

They say in their memoir on the *Nervous System and Sense Organs of the Medusae*, p. 153:

"The marginal sense-organs ('Gehörorgane') are of great interest, not to the morphologist or physiologist alone, but to the systematist as well, for they enable us to separate, on anatomical grounds, groups which were heretofore distinguishable only through their development. These are the Trachomedusae [*the Hertwigs include in the Trachomedusae both the Narcomedusae and the Trachomedusae of Haeckel*] and the campanularian medusae" (*Vesiculatae*, Hertwig).

"Previous attempts to find any structural distinction between those medusae which develop directly from the egg and those which are nurtured on campanularian hydroids have not been successful. Whatever system of organs we consider, we find more diversity within the limits of each group than between

the most similar families of the two groups. The Geryonidae unquestionably resembles the Geryonopsidae (Eirene, Eutima, and other vesiculate campanularian medusae with a long gastric peduncle) more closely than they do the Aeginadae (*Narcomedusae*, *Haeckel*) in the structure of their gastro-vascular system and reproductive organs, as well as in the shape of their swim-bell. This is especially true of the quadrate Liriope. Most of the Trachynemidae, Rhopalonema, and Trachynema, for example, bear a remarkable resemblance to the Eucopidae, with which Gegenbaur, relying on their anatomy, has united them. On the other hand, they are separated by so many structural features from the other Trachomedusae that their union with them has hitherto been warranted only by their mode of development. The marginal sense-organs ('Gehörorgane') alone furnish characteristics which enable us in every case to distinguish the Trachomedusae [*Trachomedusae* and *Narcomedusae* of *Haeckel*] from the campanularian medusae [*Vesiculatae*, *Hertwig*] without knowledge of their development."

"A craspedote medusa with sensory clubs ('auditory clubs'), with sense cells of ectodermal origin while the concretions ('otoliths') are of endodermal origin, may be referred with confidence to the first of these groups, whether the sensory clubs are free on the nerve ring or are inclosed in special vesicles. On the other hand, we may be sure that a medusa in which the marginal sense-organs are composed of vesiculated concretionary ectodermal cells and sensory cells with sensory hairs supplied from the lesser nerve-ring is a campanularian medusa, whether these organs lie in a closed vesicle or in an open pit."

This generalization is made the basis of the fundamental classification of the veiled medusae by *Haeckel*, who divides them (*System der Medusen*, p. 2) into two *Sub-legions*: the LEP-TOLINAE, or "Craspedota, either without auditory organs or with marginal vesicles, or velar auditory-vesicles with ectodermal otolith-cells; tentacles usually soft and flexible, primarily hollow; alternation with hydroid polyps probably universal," and the TRACHYLINAE, or "Craspedota, with auditory clubs or acoustic tentacles with endodermal otolith-cells; tentacles

generally stiff and inflexible, primarily solid; alternation with hydroid polyps not yet known."

The introduction of a distinction, vouched for by such high authorities, into the text-books has firmly established it, although it is by no means as absolute in nature as it is in the books.

We see that true endodermal sense-clubs occur among the "Leptolinae," in *Laodice* and its allies, and that the only difference between them and those of the *Trachylinae* is the absence of concretions.

The tentacles of many of the "Leptolinae," of many *Eucopidae*, for example, and the accessory tentacles of *Laodice* are as solid as those of any of the *Trachylinae*; and while the fixed hydroid cormus is restricted to the "Leptolinae," a hydra stage of development is, so far as our knowledge goes, common to all the veiled medusae.

McCrary pointed out in 1857 (*Gymnophthalmata*, p. 108) that the embryo of *Cunina octonaria* is "a free hydra, like the free stage of *Tubularia*," and I have shown (*The Life-History of the Hydro-Medusae*) that this is true of the *Geryonidae* and of all the *Trachylinae* whose life-history is known.

The *Trachylinae* of Haeckel are a natural group, well worthy of a distinct name, although it is important to emphasize the fact that neither in their structure nor their development do we find them fundamentally different from the other hydro-medusae.

In this connection we may note, in passing, a point in which *Laodice*, and in fact many of the campanularian medusae, resemble the *Trachylinae*. The Hertwigs have shown that the tentacles of the *Trachylinae* do not spring from the bell margin, but that they are pushed upwards by a thick pad of epithelium so rich in nettle cells that it forms a firm, cartilage-like basis for the tentacle. They also show that the endodermal axis of the tentacle is prolonged inwards above the upper surface of the circular canal. The section of the base of the tentacle of *Laodice* shown in Fig. 7 shows that it has a well-marked nettle ridge, and that the large endoderm cells of the tentacle preserve their distinctness for some distance inside

the plane of the ex-umbrella. These features of resemblance to the Trachylinae are still more marked in many of the Euco-pidae with solid tentacles.

The Systematic Rank of Laodice.

I have in this paper put such terms as "Vesiculatae," "Leptomedusae" and "Leptolinae" in quotation marks, while I have used the term Trachylinae without them.

This use of words is not capricious, although it may seem so. The "Vesiculatae" of Hertwig are the "Leptomedusae" of Haeckel, who has proposed to divide them into "Vesiculatae" and "Ocellatae"; while the "Ocellatae" of Hertwig are the "Anthomedusae" of Haeckel, which, together with his "Leptomedusae," form the sub-legion "Leptolinae." This, together with the Trachylinae, form the "Craspedota."

Every one of these terms is based on natural affinity, but no group which divorces hydroids without free medusae from their allies with free medusae is natural, nor should it be admitted into the system of zoölogy. The orders *Scrtulariae* (Agassiz), *Tubulariae* (Agassiz), and *Trachylinae* (Haeckel) are natural; but while it is often convenient to talk of hydroid medusae, as distinguished from hydroids, they should not be designated by technical terms, and I shall speak of *veiled medusae*, of *campanularian medusae*, and of *tubularian medusae*, rather than "Craspedota," "Leptomedusae," and "Anthomedusae."

Are the Trachylinae derived from one of the other groups of Hydroidea, or do the various groups converge in such a way as to indicate common descent from some remote and unknown ancestor? It is usually held that the Trachylinae are the descendants of medusae which arose on hydroid cormi, but if the common meeting-point is more primitive than any existing form, there can be no direct proof of this view. *Laodice* and the other campanularian medusae with ocelli are true campanularians; and A. Agassiz has shown that the blastostyle and medusae-buds of *Laodice* (*Lafoca*) *calcarata* are inclosed in true chitinous gonangia.

There is also a general agreement among specialists that

among the campanularian medusae the ocellate forms are the most primitive and generalized.

A. Agassiz says of the hydroid (*Lafoea*) of *Laodice calcarata* (*N. Amer. Acalephac*, 1865, p. 125): "The sterile individuals recall the Tubularians, as do in fact all the Sertularians. . . . The Medusae (*young medusae*?) of this Sertularian-like Hydro-medusarians resemble more those of the Tubularians than those of the Campanularians. The vertical diameter of the (*young*) Medusae is greater than the transverse; the bell is of moderate thickness, the abactinal part being slightly conical; the digestive cavity is short, and consists of four simple lobes, giving the actinostome the shape of a cone. When it escapes from the reproductive calycle it has only two long tentacles, two slightly developed ones, and four more hardly perceptible in the middle of the space between the four chymiferous tubes."

The Hertwigs (*Nervensystem und Sinnesorgane der Medusen*, 1878, p. 155) also hold that "we must regard the LAODICEIDAE and MELICERTIDAE as more lowly organized than the other VESICULATAE (campanularian medusae), and nearer the common type from which the VESICULATAE (campanularian medusae) on the one hand, and on the other the OCELLATAE (tubularian medusae), have been developed."

Haeckel shares this opinion, and tells us (*System der Medusen*, p. 121) that "we find among the LAODICEIDAE, in the genus *Tetranema* especially, the primitive type from which the LEPTOMEDUSAE (campanularian medusae) have been developed."

Authorities are agreed then that while the ocellate campanularian medusae are distinctly in the campanularian line they are very low down in this line, and still retain features which they have inherited from the unknown common ancestor of both tubularians and campanularians.

The occurrence among them of endodermal sense clubs is another generalized feature which they share with the Leptolinae. The *Tubulariae*, the *Sertulariae*, and the *Leptolinae* must therefore be regarded as divergent descendants of an ancestor more primitive than any known hydroid or veiled medusae, and it is clear that we cannot derive the Leptolinae from any known hydroid.

It would be interesting to follow out to its logical consequences the tacit admission by Haeckel and by the Hertwigs that we find in the structure of the medusae, *as medusae*, the data for a "system of the medusae": that they not only fall into families and orders but that they present evidence of the existence of medusae at a time before these orders were differentiated.

I cannot dwell upon this subject now although I must point out that this admission involves, as a necessary consequence, the further admission that *the veiled medusa, as a medusa, is older than the orders of veiled medusae.*

The Species or Varieties of the Genus Laodice.

As characterized by Haeckel this genus includes all campanularian medusae without marginal sense-vesicles, with four radial canals, four gonads, sixteen or more tentacles, and between them marginal clubs and cirri, or accessory tentacles.

The genus is widely distributed on both sides of the Atlantic, and A. Agassiz has described as *Laodice cellularia* (*N. Amer. Acalephae*, p. 127) a species from the Pacific, of which he says: "I am somewhat doubtful whether this species belongs to the genus *Laodice* as the examination of the tentacles could not be made sufficiently accurate to determine this point."

While it is probable that several species occur in the Atlantic, the differences between them are so slight and there is so much individual diversity that the diagnosis of the species is, in this genus, a very difficult matter.

Only one of them has been reared from its hydroid. This is *Laodice* (*Lafoea*) *calcarata* of the New England coast, which A. Agassiz has reared from a campanularian hydroid of the genus *Lafoea*.

Haeckel says of the Mediterranean *Laodice cruciata*, this delicate medusa shows so much diversity at different times of the year and at different stages of development, and its extraordinary flexibility and contractility permit it to assume such varied forms, that this single Mediterranean species has received no less than twenty different names, and every new observer has thought he had found a new species.

Haeckel himself recognizes four species. *L. calcarata* of the south coast of New England; *L. cruciata* of the Mediterranean and the Atlantic coast of Europe; *L. ulothrix* of the Canary Islands; and *L. salinarum*, from salt ditches in the vicinity of Cette in Montpellier, although he says that the difference between these species is so slight that they are scarcely more than local varieties.

I have examined three well marked varieties or species, each represented by numerous specimens. While each of them seems to be constant they differ so slightly that there seems to be no reason for giving them specific names until their whole life history has been traced.

1. Figs. 5, 6, and 7 are from a form from Woods Holl, with 100 or more large tentacles, and an equal number of accessory tentacles, and marginal clubs. The clubs have no ocelli. This is undoubtedly *Lafoea calcarata* of A. Agassiz, although he says that the accessory tentacles and clubs are irregularly distributed.

2. Fig. 4 is from a second form from Woods Holl, differing from the first only in the absence of accessory tentacles.

3. Figs. 1, 2, and 3 are from another form which I found in great abundance at Green Turtle in the Bahamas in May, 1886. The largest specimens were about an inch in diameter, with about fifty or sixty large ocellate tentacles, and an equal number of accessory tentacles, and ocellate marginal clubs. The mouth is quadrate and the lips are simple, and the distal fourth of the radiating canal is not occupied by the gonad.

Fig. 2 is from a specimen half an inch in diameter, with thirty-two primary tentacles.

In young specimens there are very few marginal clubs, and the accessory tentacles are distributed irregularly. Fig. 3 is from a specimen about a fourth of an inch in diameter, with only one club and only eight primary tentacles.

This form is very similar to *L. ulothrix* of Haeckel, from which it differs only in the distribution of the accessory tentacles and clubs, which is regular in the adult Bahama form, and irregular in *L. ulothrix*.



Fig. 1



Fig. 2

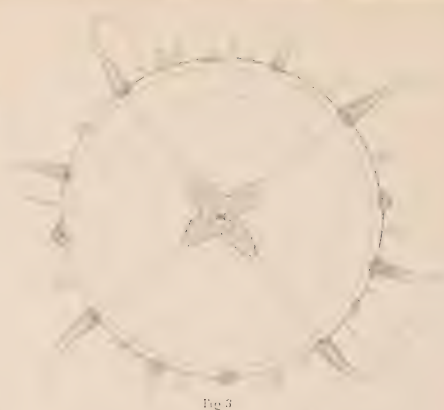


Fig. 3

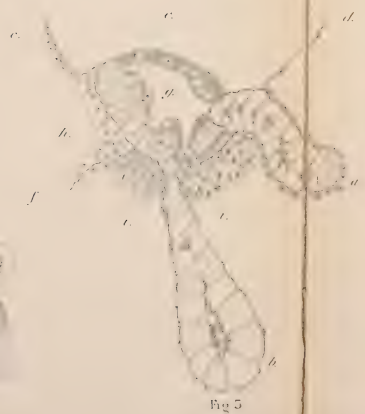


Fig. 4



Fig. 5

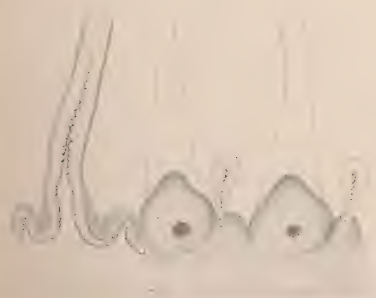


Fig. 6

THE BEHAVIOR OF THE CENTROSOMES IN THE FERTILIZED EGG OF MYZOSTOMA GLABRUM, LEUCKART.

WILLIAM MORTON WHEELER.

THE material of *Myzostoma glabrum* on which the following observations were made was collected at Naples during February and March of the current year. The work on the fecundation of the egg was brought to a conclusion in the laboratory of the Institut Zoologique at Liège, and I gladly seize this opportunity of thanking the director of the Institut, Professor Ed. Van Beneden, both for his constant and friendly guidance during my studies, and for the many favors and privileges which he so cordially bestowed upon me. A fuller account of my observations, with a discussion of the pertinent literature, will be published later.

The minute eggs of *Myzostoma glabrum* were fertilized artificially and killed in Flemming's fluid (weaker formula). The sections (5μ thick) were stained on the slide with Heidenhain's iron-alum haematoxylin, followed by a saturated aqueous solution of "Orange G."

The large germinal vesicle occupies the middle of the elliptical unfertilized egg (Fig. 1), and contains, besides the huge vacuolated nucleolus, 12 small chromosomes, each consisting of two short rods swollen at their ends. The lower portion of the egg is a mass of granular plasma; the remaining portions are found to be considerably vacuolated in sections, and to contain more or less yolk.

The head of the spermatozoön (Fig. 1) has a peculiar structure which has been overlooked by other investigators of the Myzostomida. In the living condition it presents alternating bands of different refraction, but when stained the more refractive bands form a series of chromatic discs embedded in an achromatic substance. Where the head tapers at either end the discs become smaller and more difficult to resolve. I have

counted 24 chromatic discs, and believe this to be very close to the typical number for *M. glabrum*. There are about three times as many in the spermatozoön of *M. cirriferum*, Leuck. I could detect no "Mittelstück" between the head and the insertion of the long, delicate tail. The latter has not been figured or described by preceding writers.

The spermatozoön always enters the egg at its lower, non-vacuolated pole (Fig. 1). What becomes of the tail during this process I cannot state. Somewhat later a pair of centrosomes may be observed to one side of, and obviously belonging to,

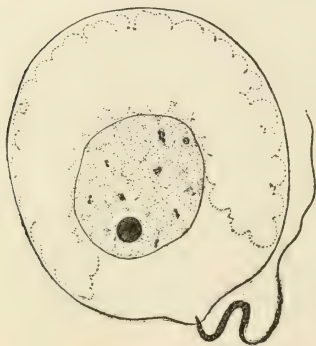


FIG. 1.



FIG. 2.

the germinal vesicle (Fig. 2). They move apart, and the archoplasm surrounding them acquires longer and more distinct radiations, which finally push in the wall of the germinal vesicle in the manner shown in Fig. 3. The wall finally fades away, the achromatic fibrils attach themselves to the chromosomes, the huge nucleolus is left out in the cytoplasm, and the spindle, which may now be recognized as that of the first polar body, moves towards the apical pole, accompanied by a mass of non-vacuolated protoplasm. This mass is continuous with the mass at the lower pole, and constitutes with it from this time forth an uninterrupted pillar running through the long axis of the egg from pole to pole. The head of the spermatozoön shortens and thickens, and the chromatic discs appear as deeply staining

nodules (Fig. 4). *The nucleolus of the germinal vesicle remains in the cytoplasm as an inert mass, gradually melting away, but not disappearing till about the 8-cell stage, when it may often be found in the largest blastomere.* This blastomere, I believe, gives rise to the entoderm. Haecker has called attention to a similar persistence of the nucleolus in the egg of *Æquorea*.¹

In the first polar body spindle each chromosome separates into its two rod-shaped halves, each half, which assumes the shape of a Diplococcus, moving towards one of the poles. At the poles the centrosomes have already divided. After the



FIG. 3.

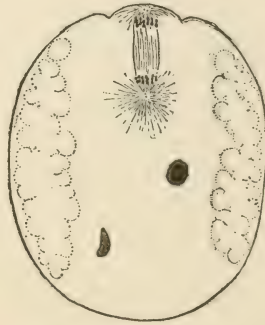


FIG. 4.

first polar body is constricted off, the second is speedily formed. While the spindle of this polar body is in the equatorial plate stage the chromosomes again split longitudinally and the centrosomes again divide, so that two centrosomes go off with the second polar body, and two remain in the egg. The first polar body often begins to divide, but, so far as I have been able to observe, its small spindle never gets beyond the equatorial plate stage. The second polar body is always fully twice as large as the first, and stains more deeply (Fig. 5). During its protrusion the interzonal fibrils of the spindle are brought together in a sheaf-like bundle at the boundary line between the cells, and there form a peculiar deeply staining plate, which

¹ *Arch. f. mikr. Anat.*, Bd. 40, 1892.

may be likened to the more extensive middle-plate in plants. This thickening persists for some time on the inner surface of the second polar body (Fig. 6).

Both the nucleus of the second polar body and the female pronucleus often consist at first of several distinct vesicles, but these soon fuse to form typical spherical or oval nuclei. While the enlarging female pronucleus is moving back from the apical pole towards the center of the egg, a mass of archoplasm containing two centrosomes may be distinguished near its lower face (Fig. 5). *I have never been able to find any traces of such*

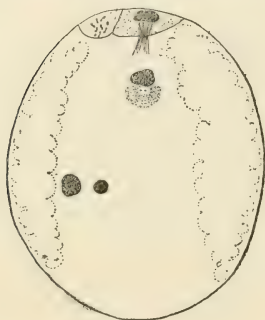


FIG. 5.

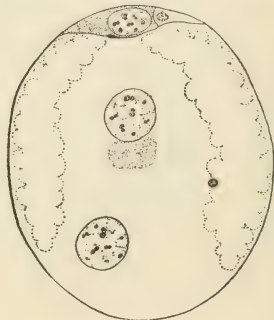


FIG. 6.

archoplasm or any centrosome in connection with the male pronucleus.

Before this time both of the pronuclei have reached exactly the same stage of development, and continue to keep accurate pace with each other. At first 12 very minute Diplococcus-like chromosomes may be made out in each pronucleus. Each chromosome increases to several times its original volume during the growth of the pronuclei, and then breaks down into a string of small karyo-microsomes. A few of the large spherical granules, however, remain as nucleoli (Fig. 8).

In many eggs of this stage I have looked in vain for any traces of archoplasm or centrosome, but whenever these structures could be brought out by means of the iron-alum haematoxylin, they were always close to the female pronucleus and

at some distance from the male. Their appearance is that of Fig. 7, where the first traces of a spindle are forming between the two centrosomes — which I take to be the two originally stationed at the inner pole of the second polar body spindle — and faint traces of radiations can be detected in the surrounding archoplasm. These radiations soon come out with startling clearness, and the centrosome of each astrosphere immediately divides (Fig. 8). Occasionally there are three centrosomes in each astrosphere like those figured by Heidenhain.¹ This stage and the ones which follow in the formation of the first cleavage

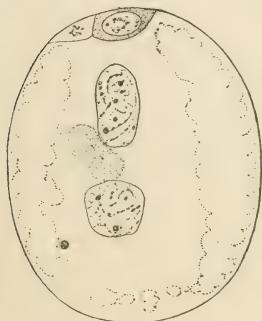


FIG. 7.



FIG. 8.

spindle have been misinterpreted by Fol.² *The centrosomes are not fusing in pairs, but already dividing in anticipation of the 4-cell stage of the ovum.*

The two pronuclei are very soon brought together, as in Fig. 9, and the first cleavage spindle is established. *In Myzostoma this spindle does not conform to O. Hertwig's law,³ but always lies at right angles to the longest axis of the often very narrow protoplasmic pillar of the egg.* The walls of the pronuclei are pushed in irregularly by the spindle fibers in much the same fashion as was the wall of the germinal vesicle in an earlier stage. They ultimately fade away, and the chromatin,

¹ Neue Untersuchungen über die Centralkörper, 1894, Taf. XXVI, Fig. 27.

² Le quadrille des centres. Arch. des Sciences Phys. et Natur., t. XXV, No. du 15 Avril, 1891, Figs. 8-10.

³ Zelle und Gewebe, p. 175.

now in the form of distinct loops made up of microsomes, is aggregated in two discrete masses in the equatorial plate (Fig. 10). The nucleoli which have appeared and developed during the growth of the pronuclei, are cast out of the spindle into the cytoplasm, where they dissolve away; the chromatin loops splitting, meanwhile, and migrating towards the poles. I estimate the number of loops, which finally arrive at each pole, at 24.

During these movements on the part of the first cleavage spindle, the egg, when viewed from the side, has the shape of a trefoil. The first cleavage plane passes obliquely from the indentation in which the polar bodies lie to one side



FIG. 9.

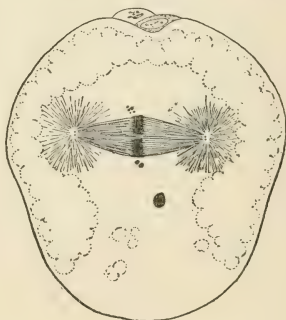


FIG. 10.

of the median lower protuberance, thus cutting off about one-third of the egg. The blastomeres become rounded off, and the 2-cell stage may be described as having a large and a small blastomere. Beard's description¹ of this and the other cleavage stages of *Myzostoma* is incorrect.

The above observations on *Myzostoma* are of a nature to restrict the generalizations which the papers of Fol,² Guignard,³ and Conklin⁴ have called forth. In *Myzostoma* there is every reason to believe that the *female* pronucleus alone is provided

¹ Mitth. a. d. zool. Staz., Neapel, Bd. V, 1884.

² *loc. cit.*

³ Nouvelles Études sur la Fécondation. Ann. des Sciences Nat. Bot., t. XIV, 1891.

⁴ The Fertilization of the Ovum. Lectures of the Marine Biol. Lab. of Woods Holl, 1894.

with centrosomes, and that only these enter into the formation of the first cleavage spindle. On the other hand, Mr. Mead's studies on the egg of *Chaetopterus* show that it is the *male* pronucleus alone which furnishes the centrosomes. I regard Mr. Mead's observations as more satisfactory than my own, for the reason that *in the Annelid both the centrosomes and their enormous radiations persist and may be followed continuously throughout the whole period of fecundation and even throughout cleavage*, whereas in *Myzostoma* there is a stage preceding the meeting of the pronuclei, when it is extremely difficult or even impossible to make out the structures in question.

In some animals, therefore, the schema proposed by Fol and Conklin appears to be considerably simplified by the suppression of the centrosome of one of the pronuclei. If the centrosomes are really permanent cell-organs whose sole and special function it is to preside over division, it is perhaps quite unnecessary that the "quadrille" should be danced in every egg, so long as the egg is sure of receiving from one or the other parent the apparatus wherewith to carry on its long series of divisions. Certainly the parthenogenetic egg is one of "Nature's experiments," demonstrating the possibility of development without a "quadrille." Moreover, the great physiological value of an organ like the centrosome is no *a priori* argument against its one-sided origin, since botanists have shown that the chromatophores and chlorophyll-bodies of plants — organs of very great physiological value to the cells in which they occur — may, during fecundation, come from one or both parents.

SOME OBSERVATIONS ON MATURATION AND FECUNDATION IN CHÆTOPTERUS PERGA- MENTACEUS, CUVIER.

A. D. MEAD.

My investigations on this annelid were begun last summer at the Marine Biological Laboratory, at Woods Holl, Massachusetts. I wish here to express my appreciation of the kind assistance given me by my friend Dr. W. M. Wheeler, and to thank Mr. George Gray, who procured for Dr. Wheeler and myself an ample amount of beautiful material. My own material having been collected for the cleavage stages, Dr. Wheeler generously turned over to me all that he had preserved for the study of maturation and fecundation.

The eggs were fixed with picro-acetic acid, and stained with Heidenhain's iron-alum haematoxylin and "Orange G."

Until the entrance of the spermatozoön the egg remains with the first maturation spindle in the equatorial-plate stage. There are nine chromosomes arranged in a circle, with eight at the periphery, and one in the center. Later, there are at each pole of the spindle, nine chromosomes, which have the same arrangement. Converging protoplasmic rays, meeting at a dark spot at either end of the spindle, are at first perfectly clear. During the later stages of karyokinesis, two centrosomes, connected by a whitish band, are clearly distinguishable at the inner end of the spindle. They gradually separate from each other. A dark spot (centrosome?) lies in the polar globule at the outer end of the spindle (Fig. 1). Meanwhile, the young male pronucleus, provided with a centrosome and radiations, approaches the animal pole, its centrosome sometimes preceding, but often following the pronucleus (Fig. 1).

Before the second polar globule is expelled the male centrosome divides, and very conspicuous fibres are seen to radiate from the surrounding dark-colored archoplasm. A single

centrosome and rays may now be seen at the inner end of the second maturation spindle (Fig. 2). In the connecting fibres there are equatorial thickenings, *Zwischenkörper* (?).

The nine chromosomes left in the egg after the expulsion of the second polar globule become vesiculated so that the female pronucleus has the form of a cluster of vesicles, in the midst of which lies the centrosome, with numerous long rays running out from it. As the female pronucleus moves back toward the male the rays become gradually less numerous. They converge to the center of the cluster in the manner represented in Fig. 3. At this time the male pronucleus is large and is provided with its two centrosomes, from which enormous rays extend to the periphery of the egg in every direction (Fig. 3). In stages like that represented in Fig. 4, the last traces of radiation connected with the female pronucleus may usually be seen. Often one can still trace the outlines of the component vesicles of the latter, though they are closely pressed together into a single oval nuclear mass.

By the time the pronuclei meet, the vesicles of the female pronucleus have apparently fused together. I can find no trace of centrosomes, or rays, connected with it, although those of the male pronucleus have become even more distinct. The two pronuclei meet, become closely applied to each other, and then the two male centrosomes, always found at the foci of two huge systems of protoplasmic rays, move far apart, greatly elongating the segmentation nucleus. In the latter, at all times, the two pronuclei may be distinguished as separate structures (Fig. 5). The chromosomes next assume their definite shape and arrange themselves in the equatorial plate. The segmentation nucleus always contains numerous nucleoli which during karyokinesis, are dropped out into the cytoplasm, where they ultimately degenerate. They are usually, if not always, found only in the larger of the two cells.

By the time the equatorial plate is formed, the centrosomes have again divided, and have begun to move apart, anticipating the cleavage into four cells. At this time also a peculiar lobe begins to make its appearance upon the lower hemisphere of the egg, always exactly opposite the polar globules. Fig. 6

represents a somewhat later stage of the egg, viewed in horizontal section. It, therefore, shows the four centrosomes, but not the lobe. The latter is represented in vertical section at still a later stage in Fig. 8.

When the chromosomes have reached a position near the poles of the spindle, each of them swells up to form a vesicle in which at first two distinct rows of granules may be seen, recalling Victor Herla's "*division secondaire*" in the centrosomes of *Ascaris megaloccephala*. Later each chromosome exactly resembles a miniature nucleus. They are separated from one another, and rays from the astrosphere may sometimes be seen in the interspaces. The two centrosomes at either pole have by this time traveled some distance apart. The rays are exceedingly clear, and extend to the periphery in all directions (Figs. 7 and 8.)

A few rays may be traced into the before-mentioned lobe at the vegetative pole. The lobe has now begun to constrict at its base (Fig. 8).

The nuclear vesicles swell still more, press against one another laterally, and constitute in each cell a disc-shaped nucleus, which later becomes spherical. Whether the vesicles actually fuse or not, I do not know.

The lobe spoken of above becomes completely constricted off and lost without leaving a scar upon the egg. It is always borne upon the larger cell and is composed almost exclusively of yolk. It is covered by the egg-membrane. Fig. 9 is from a camera drawing of an egg mounted whole, with the lobe almost completely pinched off. The lobe may always be seen connected with the egg by a more or less narrow neck during the reconstitution of the nuclei, and it always completely disappears by the time the nuclei have become spherical (Fig. 10).

It is obvious that the foregoing account of the behavior of the centrosomes is entirely unlike that of the "quadrille of the centrosomes" described by Fol, Guignard, and Conklin. I am convinced that in *Chætopterus* there is no fusion of the male and female centrosomes, and I believe that the female centrosomes degenerate after the polar globules have been expelled.

The evidence in support of my position may be summarized as follows:

(1) *By the method employed, male centrosomes, with their powerful radiations, are perfectly distinct at every step in the process, from the time of the expulsion of the first polar body up to at least the four-cell stage.*

(2) *The female centrosomes and their rays are also perfectly distinct throughout the maturation stages, are always found with the female pronucleus at first, and thenceforth gradually become fainter, until, before the pronuclei have met, not a trace of them can be seen; though the male centrosomes and rays, meanwhile, are brought out with ever-increasing clearness.*

(3) *The female centers of radiation, so long as they can be seen, never take a position in advance of the female pronucleus.*

(4) *If the female centers should join those of the male they would be obliged to make their way through the closely packed fibers of the latter; and though the female centers themselves might not be brought out by our method, they would necessarily displace the fibers of the male centers. No such disturbance in the fibers can be seen.*

In a stage like that represented in Fig. 8, just before the formation of the cell-membrane between the two blastomeres, a fusiform bundle of connecting fibers (*Verbindungsfasern*) may be seen running from one nucleus to the other. As the membrane forms there is developed in it a well-defined middle plate (*Zwischenkörper*). With the appearance of the latter the bundle of fibers becomes constricted in the middle (Fig. 10). Similar phenomena take place during the division of the two blastomeres.

In the early stages of the middle plate its component corpuscles can be distinguished (Fig. 10). In its later stages it becomes more compact and homogeneous: meanwhile the connecting fibers appear to lose their connection with the nuclei (Fig. 11).

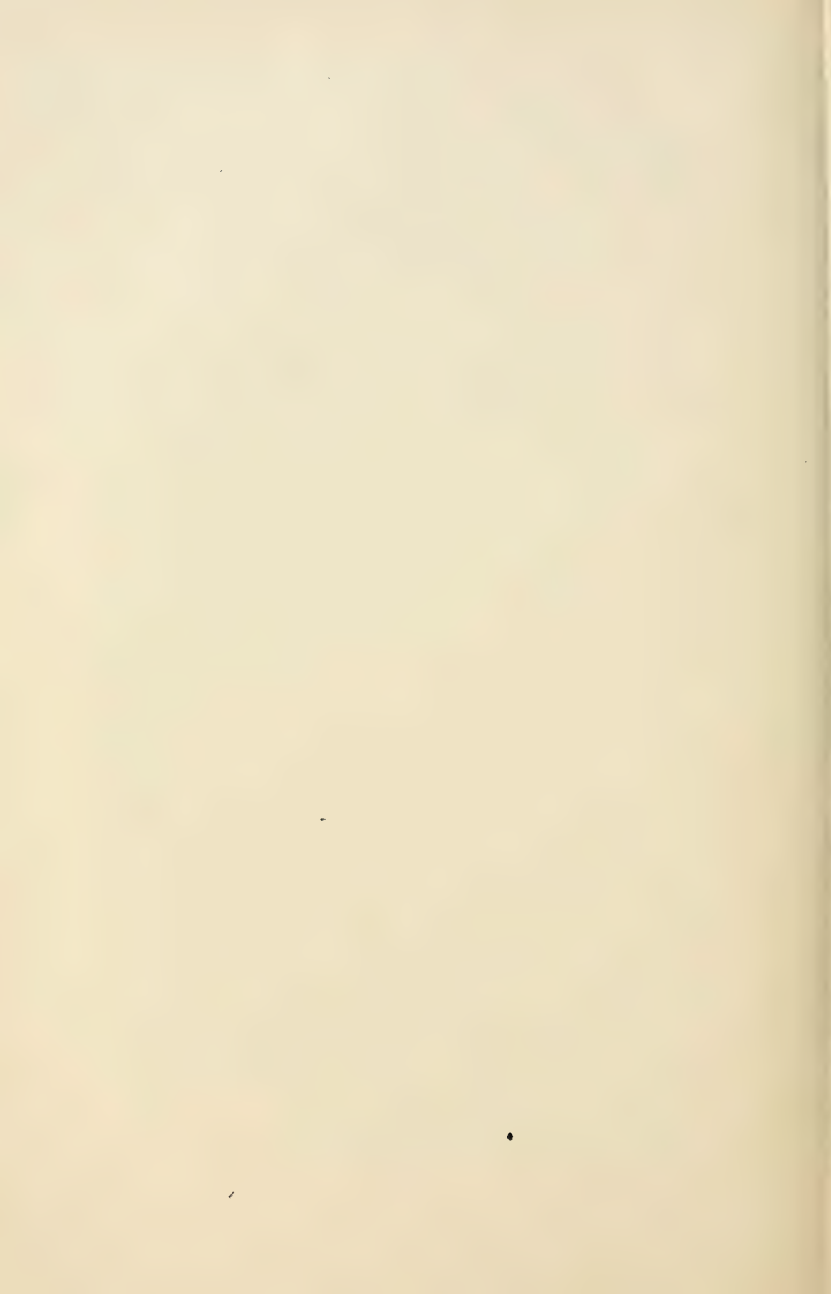
The two centrosomes at each end of the first cleavage spindle, whose origin has been mentioned above (compare Fig. 6), continue to move apart during the reconstitution of the nuclei. Ultimately, each nucleus becomes spindle-shaped,

with a centrosome at each end. The new spindles form directly, and the egg divides into four cells. Just as the new spindles arrive at the equatorial-plate stage, each of the four centrosomes divides, anticipating the cleavage of the egg into eight cells. The new centrosomes also travel apart during the reconstitution of the four nuclei (Fig. 11). In the second cleavage, as in the first, the nucleoli are dropped out into the cytoplasm in the equatorial plane (Fig. 11). *At every phase of the nucleus in the two-cell stage, the centrosomes, with radiations, are perfectly clear, and lie outside the nucleus in the neighboring cytoplasm.* This is of interest à propos of Hertwig's assertion (*Die Zelle und die Gewebe*, p. 46) "dass auch mit neueren Methoden und optischen Hilfsmitteln sich Centrankörperchen für gewöhnlich neben dem ruhenden Kern im Protoplasma der Zellen nicht nachweisen lassen."

In the four, eight, and sixteen-cell stages, the karyokinetic phenomena are the same as in the two-cell stage, as regards the behavior of the centrosomes, the nucleoli, the *Zwischenkörper*, the chromosomes, and the reconstitution of the nuclei. One can always count about eighteen nuclear vesicles at each pole of a spindle.

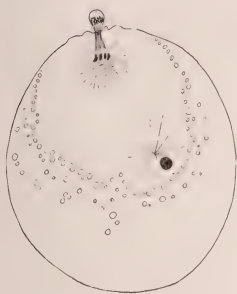
With regard to the cytogeny of *Chætopterus*, it is interesting to note that while the egg segments in the typical annelid fashion up to about sixty-four cells, including the formation of the "apical rosette" (Wilson: *Nereis*), no "cross" is formed, but the cells in question continue to divide obliquely (spirally). This annelid is, so far as I know, unique in this respect.

Nereis, *Amphitrite*, *Clymenella*, and *Lepidonotus*, are, I believe, the only annelids in which the exact origin of a part, or the whole, of the prototroch has been determined. A large part of the prototroch is formed, in all cases, by the very same cells, which cease to divide thenceforth. In *Chætopterus*, however, these cells continue to divide without interruption. Wilson showed, several years ago, that there is no prototroch in this form.

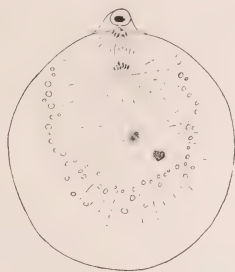




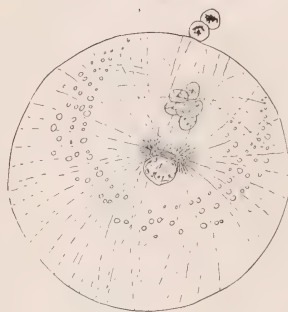
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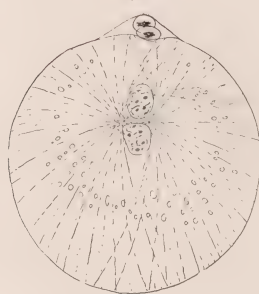
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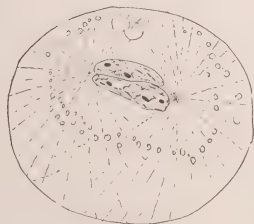
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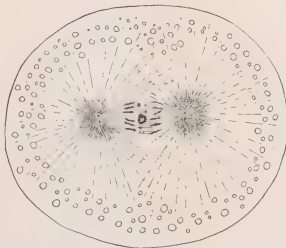
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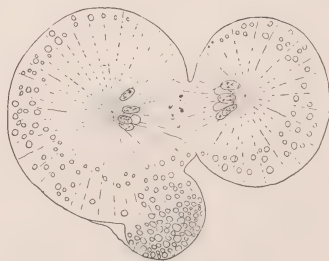
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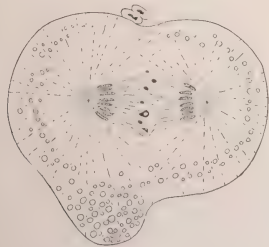
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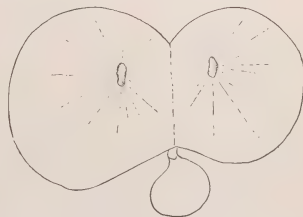
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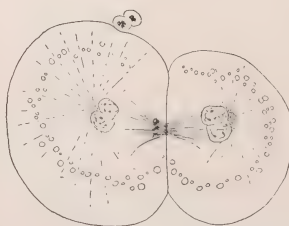
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MATURATION, FERTILIZATION, AND POLARITY IN THE ECHINODERM EGG. NEW LIGHT ON THE "QUADRILLE OF THE CENTERS."

EDMUND B. WILSON AND ALBERT P. MATHEWS.

No cytological paper of late years has aroused more general interest than that which contains Fol's remarkable account of the "Quadrille of the Centers" in the echinoderm egg. Brief and insufficiently illustrated as that paper is,¹ it contains not only very explicit statements of observed fact, but also what seems to be strong internal evidence of their accuracy. We were accordingly led to reëxamine the phenomena, not with any expectation of reaching results contrary to those of Fol, but in the hope of confirming and extending them. The investigation was begun by the senior author in the case of *Toxopneustes variegatus*, Ag., a form in which the eggs are of glass-like transparency in life, and yield brilliantly clear pictures in sections. Many of the phenomena can in this species be followed in the living egg, and important data were thus obtained regarding the paths of the pronuclei and their relations to the future cleavage-planes and to the polar differentiation of the egg. (See Part I.) In serial sections (especially after fixation by sublimate-acetic and staining on the slide by iron-hæmatoxylin, followed or preceded by Congo red, Bordeaux red, or acid fuchsin) every step in the fertilization can be followed, without a gap, from the moment of entrance of the spermatozoon up to the cleavage stages, and with a clearness such that all the essential features can easily be studied in micro-photographs (1000 diameters), of which an extensive series has been prepared. In the meantime, Mr. Mathews made a parallel and independent examination of the fertilization in another species of sea-urchin (*Arbacia punctulata*, Gray), and also in a star-fish (*Asterias Forbesii*, Verrill), the eggs of the latter form being

¹ *Anat. Anz.*, VI, 1891, and *Arch. Sci. Phys. et Nat.*, XXV, 1891.

nearly as favorable as those of *Toxopneustes*, and affording very convincing evidence. The results of this investigation agree in all essential points with those reached in the case of *Toxopneustes*; and our conclusions are therefore here presented side by side.

Our independent but concordant results are opposed to those of Fol on every essential point. *After the formation of the second polar body the egg-archoplasm soon disappears, and no egg-centrosome or egg-archoplasm ("ovocentre" as opposed to the "spermocentre") can be discovered at any subsequent period. There is nothing like a "quadrille" to be seen, save in double-fertilized eggs (Toxopneustes). The archoplasm of the first cleavage-amphiaster is developed entirely from, or under the influence of, the sperm-archoplasm ("spermocentre" of Fol), and this is derived, not from the apex of the spermatozoon, but from its base, undoubtedly from the middle-piece (Toxopneustes, Arbacia).*

It is, of course, possible that the phenomena of fertilization may differ in different species of echinoderms. The agreement between the three forms we have studied is, however, a noteworthy fact, the more striking since the eggs of *Arbacia* differ considerably from those of *Toxopneustes* in size, texture, and pigmentation, and since in *Asterias* the history of the sperm-nucleus and archoplasm differs somewhat from that of *Toxopneustes*, and in such wise as greatly to strengthen the negative evidence. Comparison of a large series of serial sections of these three forms fixed by various reagents (sublimite, sublimite-acetic, chromic acid, chrom-acetic, Flemming's fluid, Hermann's fluid, picro-sublimite, and Fol's picro-osmic mixture) leads to the conviction that Fol's methods were untrustworthy and his results open to question. Whether so experienced an observer can have been misled by double-fertilized eggs, which give a simulacrum of a "quadrille" (see Fig. 4, D), may be left an open question.

It is proper to add that in the following account the senior author is alone responsible for Parts I and II (*Toxopneustes*), and Mr. Mathews for Part III (*Arbacia, Asterias*).

I. TOXOPNEUSTES VARIEGATUS.

A. OBSERVATIONS ON THE LIVING EGG.

1. *Axial relations. Paths of the Pronuclei. Polarity.*

The large germinal vesicle occupies an eccentric position, and the polar bodies are formed (in the ovary and before fertilization) approximately at the nearest point of the egg-periphery. After the extrusion of the second polar body the egg-archoplasm entirely disappears, while the egg nucleus is re-constituted as a well defined spherical vesicle, which remains near the surface in a very markedly eccentric position corresponding with that of the original germinal vesicle.¹ The axis passing through the egg-nucleus, therefore, corresponds with that passing through the germinal vesicle, and the point at which the polar bodies are formed (Fig. 1).

The spermatozoön may enter at any point with reference to this axis. There is no pre-formed "cone of attraction," but as the spermatozoön enters the egg, a protoplasmic prominence of the vitellus appears at the point of entrance and inside the vitelline membrane. This structure, which may be called the "entrance-cone" ("*cône d'exsudation*" of Fol), persists until about the time the pronuclei unite, and thus forms an unmistakable orienting point for the observation of the sperm-track.

The vitelline membrane, formed instantly after attachment of the spermatozoön, carries out with it the tail of the spermatozoön attached to its outer surface, and only the nucleus and middle-piece enter the egg. About two or three minutes after the first contact an aster appears near the point of entrance, and in some cases the sperm-nucleus may be seen beside it. Sperm-nucleus and sperm-aster, the latter rapidly growing, now move inwards, and in 5 to 8 minutes (temperature 27° C.) unite with the egg-nucleus which advances to meet them, often undergoing considerable changes of form during its progress.

¹ This was determined by keeping several eggs under continuous observation for several hours. In every case observed the egg-nucleus remained in the immediate neighborhood of the polar bodies. It is possible that it may ultimately wander from this position to other parts of the egg, but no evidence of such wandering was observed.

The paths of the pronuclei vary extremely according to the point at which the sperm enters (Fig. 1). The entrance-path of the sperm is nearly, though never quite, radial, and the copulation-path forms but a slight angle with it. That of the egg-nucleus may be in any direction from its original position (even outwards toward the periphery), and varies with the entrance-point of the sperm. *The meeting-point of the pronuclei is without constant relation to the egg-axis*, or the original position of the egg-nucleus. It is seldom at the actual center of the egg. After union of the pronuclei the segmentation-nucleus gradually moves to a position near but *not at* the center of the egg, and in this eccentric position the first cleavage-amphiaster is subsequently developed, the first cleavage-furrow invariably appearing first on that side nearest the amphiaster, and extending thence around and through the egg. The eccentricity persists on the same side in the 2-celled and 4-celled stages, and the later history of the egg shows that *the micromeres of the 16-cell stage are formed at that pole of the egg opposite to the eccentric segmentation-nucleus*.¹ In other words, *the eccentricity of the cleavage-nucleus marks the definitive polarity of the egg*.

In view of this fact it might have been expected that the eccentricity of the cleavage-nucleus would be in the same radius with that of the egg-nucleus. Such, however, is rarely the case. It is a remarkable fact (illustrated by the diagrams in Fig. 1, which are selected from a large number of observed cases), that *the eccentricity of the cleavage-nucleus has no constant relation to that of the egg-nucleus, and may be in any radius of the egg*. In other words, *the definitive egg-axis (polar axis) may form any angle with the original axis passing through the egg-nucleus*. This fact, accurately determined in a large number of cases, leaves no escape from the following alternative: If we assume the polarity of the egg to be pre-determined from the beginning, we must admit that the polarity determines the position of the segmentation-nucleus, but is without influence on that of the egg-nucleus before fertilization, so that the latter may wander to any position. If, on the other hand, the

¹ Cf. Morgan, *Anat. Anz.*, IX, 1894.

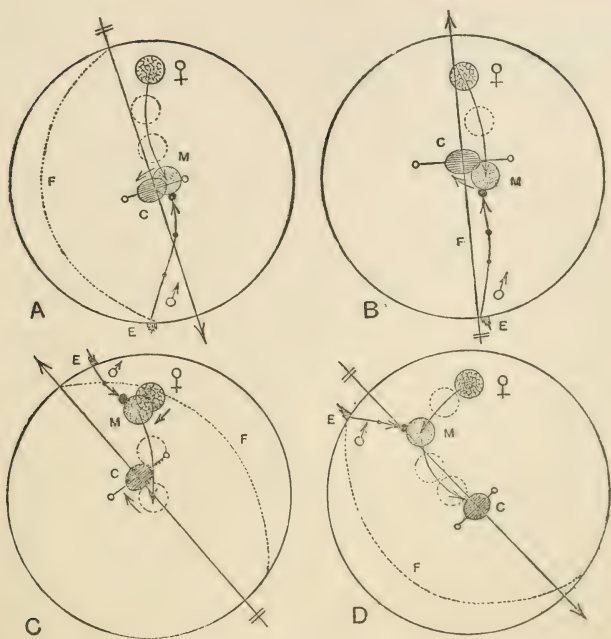


FIG. 1. Diagrams from successive camera drawings of the living eggs of *Toxopneustes*. In all, about seventy such cases have been continuously observed. The original position of the egg-nucleus is shown by the circle containing a reticulum marked ♀. *E*, the entrance-point of the spermatozoon and the entrance-cone; *M*, meeting-point of the pronuclei (egg-nucleus dotted); *C*, cleavage-nucleus (with parallel lines) in its ultimate position; the subsequent axis of the cleavage-amphiaster is shown by the line passing through the nucleus, the poles of the spindle by small circles at its ends.

The paths of the pronuclei are indicated by the curved arrows, successive positions of the egg-nucleus by dotted circular outlines, those of the sperm-nucleus by black dots. The definitive egg-axis is shown by the straight arrow, the point of which is turned toward the pole opposite to the micromere-pole. The dotted line (*F*) indicates the plane of first cleavage (in *B* it is vertical, coinciding with the arrow indicating the egg-axis).

A and *B* are diametrically opposed in polarity, though agreeing closely in other respects. *C* and *D* are also diametrically opposed in polarity, but differ considerably otherwise.

definitive polarity is not primordial, but is induced by causes operating at the time the segmentation-nucleus takes up its position, then it is brought into the category of epigenetic phenomena.

2. *Fertilization and Cleavage in the living Egg.*

When the pronuclei unite (7–8 m.) the rays of the sperm-aster extend far out into the vitellus, often nearly to the periphery. The central body of the aster then becomes much enlarged (“aureole phase” of Fol) and divides into two halves, which diverge to opposite poles of the segmentation-nucleus (12–16 m.). The latter meanwhile slowly proceeds to its definitive eccentric position. A long pause now ensues, during which the astral rays become less marked, and finally almost disappear, while the nucleus considerably increases in size (20–30 m.). At 25–30 m. the nuclear membrane fades away quite suddenly, disappearing first at the poles nearest the archoplasm-spheres, and wholly fading away within two or three minutes. Astral rays now again extend themselves from the archoplasm-spheres, a beautiful amphiaster is developed (35–40 m.), and the first cleavage takes place (45–50 m.).

The axis of the cleavage-spindle is always nearly at right angles to the copulation-path; and since the angle between copulation-path and entrance-path is small *the plane of first cleavage is, in the great majority of cases at least, approximately through the entrance-point of the sperm* (Fig. 1).¹

B. FERTILIZATION AND CLEAVAGE IN SECTIONS. TOXOPNEUSTES.

I. *Structure of the first Cleavage-amphiaster.*

The history of the archoplasmic structures may best be considered by beginning with the first cleavage-amphiaster at a stage corresponding with Fol's Fig. 10. In general appear-

¹ This was determined by the continuous observation of about seventy cases. In only one case was a deviation as great as 45° observed; it was rarely as great as 15°. This agreement proves that no serious error can have been committed regarding the axial relations, described above, through accidental rolling of the eggs.

ance the amphiaster (Fig. 3, *D*) corresponds closely with Fol's figure, the center of each aster being occupied by a clear sharply circumscribed archoplasm-sphere ("*astrosphère*" of Fol). This sphere is described by Fol as consisting of a clear liquid or jelly in which is suspended a single centrosome ("*astrocentre*"), the latter having arisen through the fusion of the paternal and maternal "demi-centers." *This description is totally inapplicable in the case of Toxopneustes.* The central sphere here consists of a distinct *reticulum* with thickened nodes, staining bright red after iron-haematoxylin and Congo red or acid fuchsin, while the astral rays are blue. The appearance of the reticulum varies somewhat according to the mode of fixation. After Flemming's fluid the fibres are extremely distinct but very delicate. After sublimate or chromic acid the nodes are much coarser and the fibres less distinct. Sublimate-acetic (the best reagent for the archoplasmic structures in general) gives an effect intermediate between the two foregoing methods. Picro-osmic has a very destructive action, the reticular substance being disorganized and broken up into clotted granules, often leaving the "*astrosphere*" quite empty, or containing one or more irregular clumps closely similar to those described by Fol as "*centrosomes*." In *Toxopneustes* these are unquestionably artefacts — mere fortuitous groups of granules, inconstant both in number and size. In well-preserved material the thickened nodes of the reticulum appear as granules (clearly shown as such in photographs) uniformly distributed through the astrosphere, and *there is absolutely nothing to be identified as a centrosome.*

During the anaphase the central sphere rapidly increases in size and becomes much looser in texture (Fig. 4, *A*), and as the daughter-nuclei are reconstituted the astral rays become less distinct, the aster finally appearing as a large blue granular mass almost amoeboid in outline and traversed by faint rays. The nucleus is withdrawn into the interior of this mass and is pressed against the red reticular mass, which flattens down upon it like a cap (Fig. 4, *B*) and in some preparations seems nearly to surround the nucleus. The egg meanwhile divides into two. The central archoplasm-mass now divides (in a plane

passing through the former spindle-axis) into two halves which place themselves at opposite poles of the nucleus, and this is followed by a division of the surrounding blue mass (Fig. 4, *C*). The central masses (still staining red and surrounded by the blue granular mass, traversed by faint rays) persist in this position throughout the 2-celled stage and form the centers of the second cleavage-spindles, which resemble the first in all respects.

It is clear from these facts that the central reticular erythrophilous sphere is identical with an "attraction sphere," but it contains no differentiated centrosome or even a "central group." It will now be shown that *the archoplasm-spheres are derived by direct descent from the sperm-archoplasm (and ultimately from the middle-piece) without the participation of a visible "ovocentre."*

2. Entrance of the Spermatozoön and Copulation of the Pronuclei.

The head of the spermatozoön (Fig. 2, *A*) is lance-shaped, the nucleus staining intensely blue, while the middle-piece is red. (There is also a very minute erythrophilous body at the tip, easily demonstrable in fresh material, but not visible after fixation.) It enters the egg point first (Fig. 2, *B*), but almost immediately afterwards rotates through about 180° , so that its base is directed inwards (Fig. 2, *B-F*). The middle-piece cannot be distinguished in *Toxopneustes* at first (*cf.*, however, *Arbacia*) since it stains like the surrounding cytoplasm. During the rotation, however, a small aster appears at or near the base of the nucleus (Fig. 2, *D*), and this is undoubtedly derived from the middle-piece. The aster rapidly grows as the sperm advances, its rays finally traversing nearly an entire hemisphere of the egg. The central body of the aster is occupied by an ill-defined granular mass, staining red, while the astral rays are faintly blue. The sperm-aster therefore agrees on the whole with the cleavage-aster, but the central mass does not yet show a definite reticular structure, and the color-contrast with the rays is less marked.

During its inward progress the aster is in advance of the sperm-nucleus, and as a rule lies somewhat to one side of it.

In many cases, if not in all, it is the aster that first comes in contact with the egg-nucleus, against which it becomes closely pressed (Fig. 2, *H*). The sperm-nucleus, as it follows the aster, loses its lance-shaped outline, becomes oval, swells some-

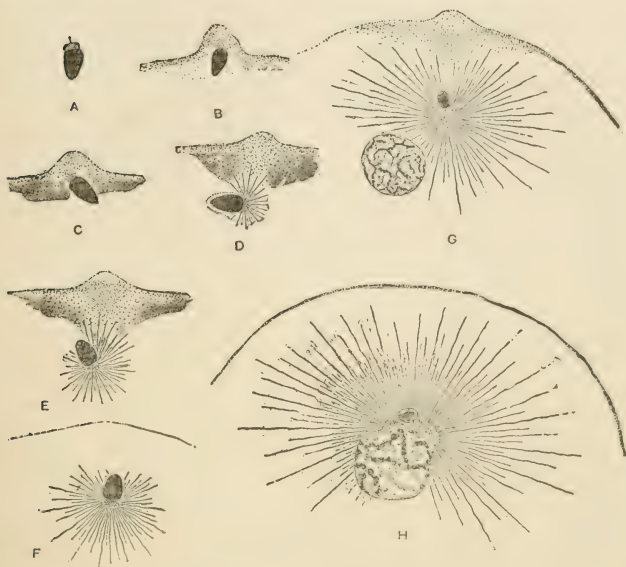


FIG. 2. Entrance and further history of the spermatozoön up to its conjugation with the egg-nucleus in *Toxopneustes* (this and the following figures from camera drawings of sections after fixation with sublimate-acetic, stained on the side with iron-haematoxylin and Congo red). *A*, free sperm-head, with granular middle-piece; *B*, *C*, *D*, *E*, *F*, successive stages in the penetration and rotation of the sperm-head, and the development of the sperm-aster; *G*, pronuclei just before union; *H*, union of the pronuclei and growth of the sperm-aster; two sperm-heads attached to the egg-periphery.

what, and finally comes into contact with the egg-nucleus, beside the archoplasm-mass (Fig. 3, *A*). The central mass now stains bright red with acid-fuchsin, has a definite though irregular outline, and a slightly vacuolated appearance. The archoplasm-mass rapidly enlarges and is flattened against

the nucleus, one side of which it covers like a cap (Fig. 3, *A*). In this stage ("aureole phase" of Fol) the "quadrille" should occur, but not the least sign of centrosomes can anywhere be discovered. Comparison of picro-osmic specimens at this stage with those fixed by sublimate-acetic shows that the clear space figured by Fol (*e.g.*, in his Figs. 4-7) is an artefact, caused by the destruction of the archoplasm-mass and the shrinkage of the nucleus.

The central mass now draws apart in the middle and divides into two halves which lie at opposite poles of the cleavage-nucleus (Fig. 3, *B*). Meanwhile the sperm-nucleus enlarges still further, becomes closely pressed against the egg-nucleus so as to assume a lens-shape, and its substance becomes reticulated. The boundary between the two pronuclei then fades away, and they ultimately fuse completely to form a single reticular "segmentation-nucleus," in which it is impossible to distinguish between the maternal and paternal chromatic substances (Fig. 3, *C*).

3. *Formation of the Cleavage-spindle.*

During the ensuing pause the astral rays become much shorter and less conspicuous, the aster becoming greatly reduced in size. The red central mass, however, does not diminish, and at this period its reticular character first becomes clearly apparent (Fig. 3, *C*).

About 30 min. after fertilization the rays again become conspicuous, and at this stage in favorable specimens the future spindle-fibers may be clearly seen to penetrate into the segmentation-nucleus at either pole, where the membrane fades away. The remainder of the nuclear membrane now rapidly disappears, and the peripheral spindle-fibers appear exactly in its place, so that the spindle as a whole appears to be formed by a direct transformation of the achromatic nuclear substance (including the membrane). Meanwhile the nuclear reticulum is transformed into the chromosomes, and the first cleavage-spindle is formed (Fig. 3, *D*).

To sum up: *The central archoplasm-sphere ("attraction sphere") of the cleavage-amphiaster is derived by direct and*

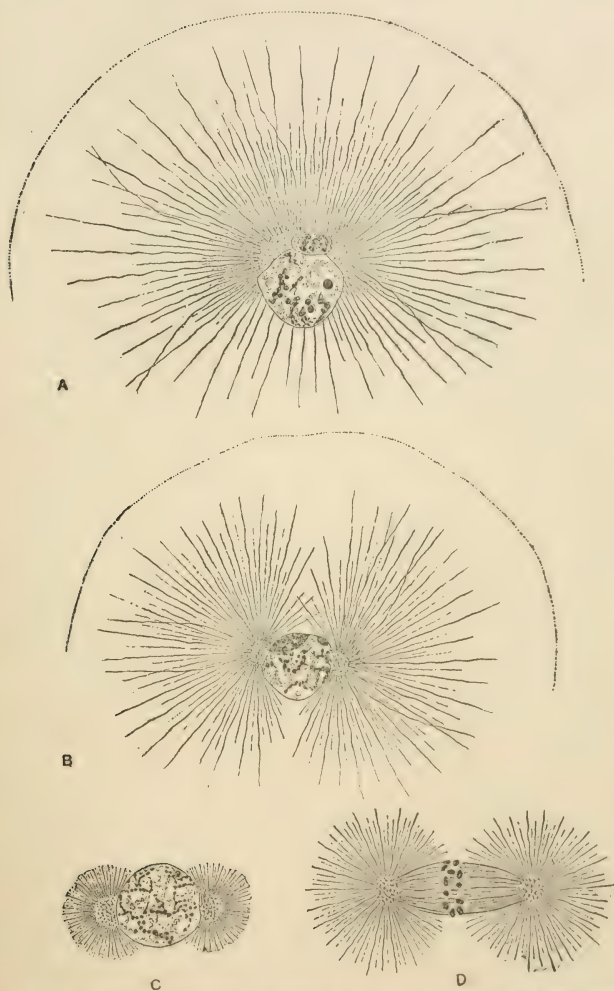


FIG. 3. Fusion of the pronuclei, fission of the archoplasm-mass, and formation of the cleavage-amphiaster in *Toxopneustes*. A, "aureole-phase" (cf. Fol, Fig. 4); B, double-aster (cf. Fol, Fig. 5); C, pause (cf. Fol, Fig. 8); D, early cleavage-amphiaster (cf. Fol, Figs. 9 and 10).

unbroken descent from the central mass of the sperm-aster without visible participation of an egg-center. There is no centrosome save as an artefact. The sperm-aster is derived from the middle-piece, which like the archoplasm-sphere at every stage is erythrophilous.

II. THE PSEUDO-QUADRILLE IN DISPERMIC EGGS OF TOXOPNEUSTES.

The most careful study of sections at every stage of the normal fertilization gives absolutely no evidence of the existence of an egg-aster, an egg-centrosome, or a copulation of centrosomes. In double-fertilized eggs, however, a conspicuous pseudo-quadrille is enacted that affords important indirect evidence. Polyspermy may take place both in ripe and unripe eggs. In the latter case (eggs with germinal vesicle) the sperm-head penetrates only a short distance, does not unite with the egg-nucleus, and no aster is developed. In the former case (after extrusion of the polar bodies) each sperm-nucleus is accompanied by an aster that ultimately divides into two, forming a sperm-amphiaster, as is normally the case in *Asterias* (*vide infra*). In dispermy (very common in *Toxopneustes*) both sperm-nuclei copulate with the egg-nucleus, the three nuclei fusing completely together. Meanwhile, a symmetrical tetraster (Fig. 4, *D*), is developed by union of the two sperm-amphiasters, and the egg ultimately divides into four at the first cleavage¹ (as described by Hertwig and Driesch).

It has already been suggested that Fol may possibly have been misled by such cases of dispermy. Waiving this point, however, it may be pointed out that if an egg-centrosome were present in *Toxopneustes* the tetraster of dispermic eggs would result from the combination of *six centers* instead of four, and it is hard to conceive how a symmetrical division (such as actually occurs) could take place. Beside this indirect negative evidence, should be placed the additional fact described above, that in normal monospermic fertilization *the sperm may*

¹ This process was repeatedly observed in the living egg, and nearly every stage of it has been carefully studied in sections.

enter at any point and the first cleavage passes through or near this point. In the absence of a "quadrille" this fact is easily comprehensible, but very difficult to explain if a copulation of

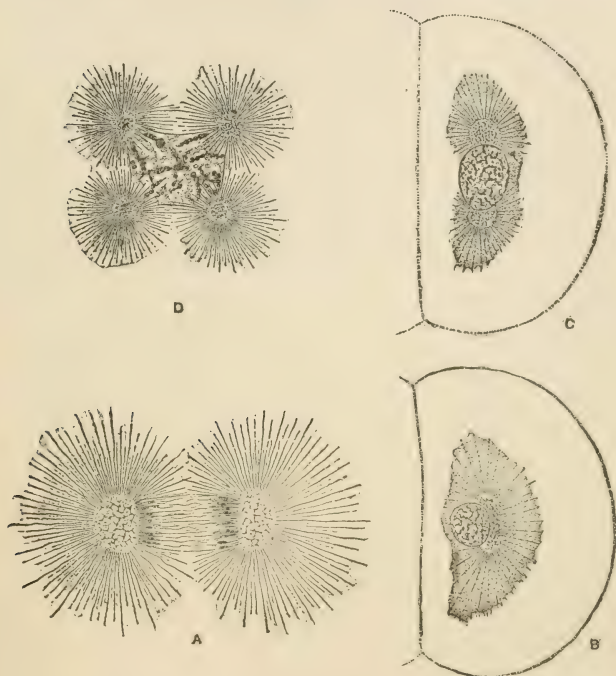


FIG. 4. *Toxopneustes*. A, cleavage-amphister in the anaphase; B, daughter-nucleus, with archoplasm-mass, soon after division; C, "resting-nucleus" of 2-celled stage, after fission of the archoplasm; D, cleavage-nucleus of double-fertilized egg, with four asters, at a stage a little later than Fig. 3, C.

centrosomes really occurs. It will hardly be denied that this double confirmation by circumstantial evidence of the results of direct observation gives a very strong case against the "quadrille" in *Toxopneustes*, and justifies a skeptical view of Fol's conclusions.

III. THE MATURATION AND FERTILIZATION OF *ASTERIAS FORBESII*. THE FERTILIZATION OF *ARBACIA PUNCTULATA*.

A. THE MATURATION OF *ASTERIAS FORBESII*.

The following description of some of the phenomena of the ovarian maturation of the starfish egg is based upon the study of a single ovary, which, fortunately, exhibited clearly the various stages of ripening until the complete formation of the first polar spindle. Owing to the difficulty of obtaining ovaries in maturation it has been impossible to establish these observations by the study of other similar specimens; but from eggs matured artificially, either by the action of sea-water or by vigorous agitation, it has been possible to confirm most of the essential facts and to extend the study through the whole process of maturation.

The eggs and ovaries throughout have been fixed in corrosive sublimate-acetic solution, a fluid which seems to yield particularly fine fixation of this material; and sections have been stained upon the slide by iron-haematoxylin, followed either by Congo red, Bordeaux red, or, in a few cases, by acid fuchsin. By this method, and in such material, the origin of the centrosomes, the history of the chromatin, and the origin and fate of the nucleolus may be followed with great clearness. Of the chromatin and nucleolus many novel things have been learned, which are reserved for a more detailed description. The origin and fate of the centrosomes are, in a paper of this nature, of particular interest, and their history is accordingly given at some length.

The artificial maturation of the echinoderm egg by rapid agitation, first pointed out by Morgan,¹ is a matter of such interest that I present the following observations in confirmation of his result. It will be found on placing unripe eggs, after a vigorous shaking, in sea-water, that a large proportion of them now extrude the polar globules, and the first globule often again divides. Such eggs, when unfertilized, do not, however, and could not be induced to, develop further, clearly

¹ *Anat. Anz.*, IX.

indicating that the action of the sperm upon the egg depends upon the introduction of a substance into the latter. In one case only twelve per cent of eggs matured when unshaken, whereas over fifty per cent were induced to mature by a little agitation; and, had the shaking been more violent or more prolonged, similar experiments upon other eggs prove that the proportion would have been increased. Sections of these eggs show that the most noticeable result of the operation is the rupture of the membrane of the germinal vesicle, and this appears to be essential to maturation. This tearing of the membrane permits the escape of nuclear matter into the cytoplasm, and there is evidence that a substance is at this time set free which, either directly or indirectly, forms the centrosomes. In ovarian normal maturation the wall of the germinal vesicle is dissolved, and finally breaks at a point nearest the surface of the egg. At this point the centrosomes are invariably developed. In eggs matured artificially without the aid of shaking the membrane becomes first wrinkled where it lies nearest the surface of the egg, and here, also, the centrosomes are formed.

1. *Origin of the Centrosomes.*

The fate of the centrosome derived from the egg mother-cell has not been accurately determined on account of the minuteness of the elements. During the skein stage of the chromatin of the very young egg, however, a small cytoplasmic accumulation like a cap is generally present at one end of the nucleus. This resembles the archoplasm, or "Nebenkern," as often figured. Whether it be the remnant of the spindle or not, it bears no constant relation to the place of formation of the nucleolus, and quickly disappears. No centrosome is thereafter to be found until the time of maturation.

At maturation the centrosomes are first accurately to be distinguished as two (at a very early stage apparently one) deeply staining, small, but distinct and characteristic, granules lying side by side either in the nuclear membrane or immediately without it, and invariably on that part of the vesicle nearest to the surface of the egg. Occasionally one of these

granules appears before the other, and migrates some distance from the nucleus before the second appears. In cases where they both lie clearly outside of the nucleus, the nuclear membrane is invariably broken behind them. Although similar granules may be seen within the vesicle, they cannot be identified as centrosomes, if they be such, on account of the lack of archoplasm or radiations about them.

When the granules first appear the archoplasm surrounds them as a faint and minute halo. At a little later stage, however, it becomes clearly distinct (see Fig. 5, *A*), and, as the centrosomes move outward from the nuclear wall, it rapidly grows, while radiations appear about it. The archoplasm-mass divides into two portions, with a centrosome in each (see Fig. 5, *B*), and these gradually move apart as a central spindle develops. The amphiaster now lies tangentially along the top of the nucleus. The nuclear wall has already disappeared in this vicinity; astral rays grow down into the nucleus; and certain irregular agglomerations of chromatin lying at the upper part of the germinal vesicle pass into the position of the equatorial plate of the spindle. (One constituent of the chromatin, by far the larger part, is converted into cytoplasm. No persistence of chromosomes occurs.) The spindle then rotates and takes up a radial position. At this time the single centrosome has divided into two or three minute granules lying in a spherical archoplasm which stains a bright vermilion in Congo red (see Fig. 5, *C*).

The chromatic masses of the spindle now arrange themselves into seventeen (?) double (quadruple?) chromosomes. The first polar body carries with it seventeen (?) double chromosomes and one of the asters. The outer centrosome of the second polar spindle is formed at the "Zwischenkörper" of the first, and the second polar body removes this and seventeen (?) single chromosomes, leaving in the egg an archoplasm containing one or two minute centrosomes and seventeen (?) single chromosomes. The egg-nucleus re-forms, as described by Hertwig and Fol, as a group of four or five little vesicles, which later fuse into one (see Fig. 5, *D*). The centrosomes disappear during this process, and the archoplasmic rays, which persist

for a time after the re-formation of the egg-nucleus, gradually disintegrate, and no trace of centrosome or radiations about the egg-nucleus is subsequently to be found.

The appearance of the centrosomes within the wall of the vesicle; the fact that the membrane is clearly fractured behind them; the minuteness of the archoplasm as they first appear, and its growth as they move outward, indicate that the centro-



FIG. 5. *Asterias*. *A*, origin of two centrosomes in the membrane of the germinal vesicle. Archoplasm appearing about them.

B, a later stage than *A*. Large archoplasms, with centrosome in each, now beginning to separate.

C, first polar spindle after rotation. Two centrosomes in each sphere. The chromosomes are still irregular in shape.

D, formation of the egg-nucleus as five small vesicles, surrounded by the archoplasmic radiations of the second polar spindle.

E, The sperm of *Asterias* before penetration, showing tip and middle-piece.

F, the entrance-cone of *Asterias*.

G, beginning of the sperm-star in *Asterias*.

somes of the egg are derived at maturation either from the interior of the germinal vesicle or from its wall, and escape from it on the rupture of the latter.

B. FERTILIZATION OF *ASTERIAS FORBESII*.

The sperm enters either ripe or unripe eggs at any point of the periphery, and in ripe eggs an attraction-cone such as is figured by Fol is formed. In unripe eggs many sperms enter, no vitelline membrane appears, the archoplasm remains insignificant, and either precedes or follows the sperm-head until the latter finally arrives at the germinal vesicle-wall, where it comes to rest. In ripe eggs dispermy is common, but not normal. The sperm enters these eggs before or during maturation, but the sperm-aster remains insignificant until the disappearance of the wall of the germinal vesicle (see Fig. 5, *G*). Owing to the difficulty of orienting the sperm-head after penetration, it has been found impossible to say, with certainty, whether the sperm-aster develops from the tip or base of the sperm, or whether the latter rotates or not (see Fig. 5, *F*). Before penetration the deeply staining sperm-nucleus is seen to have in front of it a red-staining tip, and behind it a red-staining middle-piece (see Fig. 5, *E*). In ripe eggs the undivided sperm-star precedes the sperm-head as it penetrates.

While the polar bodies are forming, the sperm star is developing and, at about the time of formation of the second polar spindle, or later, divides (see Fig. 6, *A*). After division the stars grow rapidly, the archoplasm-mass becomes very large, and in favorable sections is seen to consist of a reticulum, the nodes of which in less favorable preparations appear to be coagulated into one, two, three, or more granules like centrosomes (see Fig. 6, *B*). From the periphery of these spheres conspicuous rays develop, penetrating the egg in all directions. There is thus formed a large sperm-amphiaster of which one aster precedes and the other follows the sperm-nucleus during its further course. This is in striking contrast to the process in *Arbacia* and *Toxopneustes*. Meanwhile the sperm-nucleus has become vesicular, and at the time of conju-

gation is a reticulated vesicle but little smaller than the egg-nucleus. (Contrast *Arbacia* and *Toxopneustes*, Fig. 6, D.)

The sperm-nucleus and its amphiaster reach the center of the egg or its vicinity before the formation of the egg-nucleus,

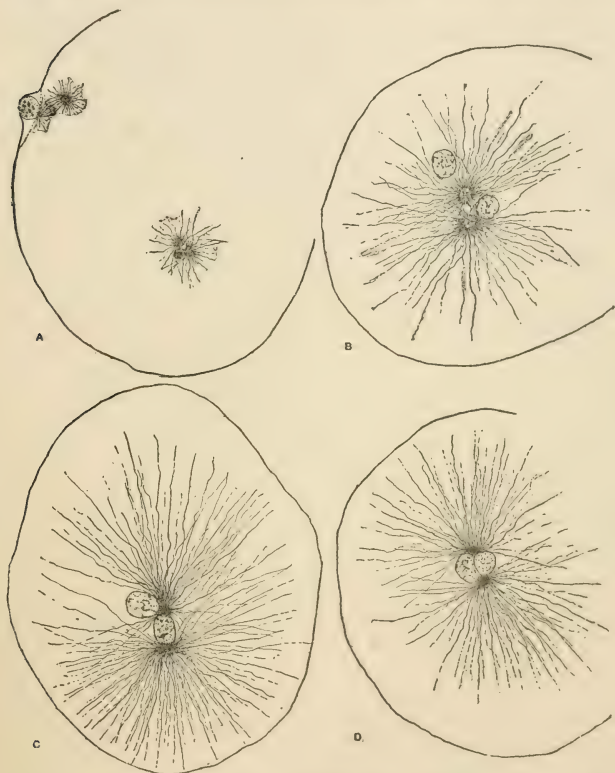


FIG. 6. *Asterias*. A, sperm-star dividing during formation of second polar spindle. Sperm-nucleus becoming vesicular.

B, sperm-amphiaster formed in center of egg. Egg-nucleus without radiations about it advancing to meet sperm-nucleus. Reticular structure of archoplasm.

C, meeting of egg-nucleus and sperm-aster.

D, conjugation of two nuclei. Sperm-amphiaster converted into first segmentation-amphiaster.

and always advance from this point to meet the egg-nucleus. This is, perhaps, the reason why in *Asterias* the first cleavage is constant, passing through the polar globules, or very near them, although the sperm enters the egg at any point. If this is so, it obviates the apparent contradiction between *Asterias* and *Toxopneustes*, that in the former the egg-axis coincides with the first cleavage-axis, while in the latter it may or may not. The two axes in *Asterias* coincide because the axis of conjugation of the two nuclei is very near the egg-axis.

The egg-nucleus re-formed, advances to meet the sperm-nucleus, and as it moves the radiations about it disappear. The front sperm-aster comes in contact with the egg-nucleus first (see Fig. 6, C), moves to one side, and the two nuclei come into apposition. The axis of the sperm-amphiaster now traverses the plane of junction of the two nuclei and its position corresponds with that of the first cleavage-amphiaster (see Fig. 6, D).

The division of the sperm-aster previous to the meeting of the two nuclei is an important point of difference from the procedure in Toxopneustes and Arbacia, and gives additional reason for doubting the occurrence of any quadrille such as is figured by Fol. By a division of the asters before conjugation the formation of the first segmentation-amphiaster directly and wholly from the sperm-amphiaster is clearly seen. If there is a quadrille it is necessary to suppose that the egg-centers have no radiations about them, in which case no observer could identify them; that they are so small as not to be distinguished after the closest search; that before conjugation the egg-centers divide and take up a proper position on the egg-nucleus to meet the sperm-asters, which have already assumed their definite position; and that the egg-centers after conjugation are no longer to be discerned in the segmentation-asters, since there is in these no discoverable trace of the two centrosomes as figured by Fol.

Having come into apposition, the two nuclei, now occupying a slightly eccentric position in the egg, fuse completely and indistinguishably to form the segmentation-nucleus. The large radiations about the asters become more difficult of demonstra-

tion, and the segmentation-spindle is soon formed. The archoplasms of this spindle are of the same reticular structure described for *Toxopneustes* and *Arbacia*.

C. FERTILIZATION IN *ARBACIA PUNCTULATA*.

The fertilization in *Arbacia* presents a striking similarity to that in *Toxopneustes*, but on account of differences in texture,

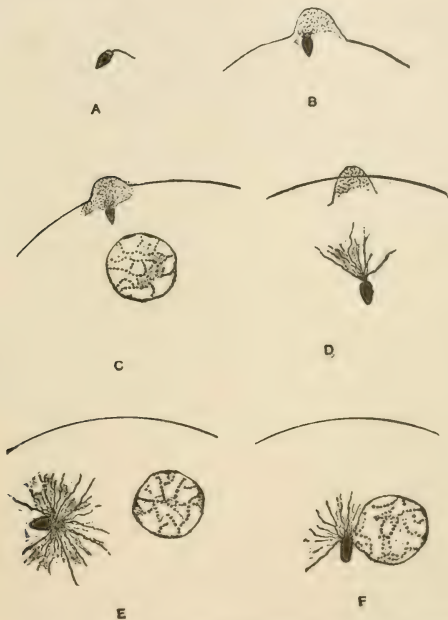


FIG. 7. *Arbacia*. A, sperm of *Arbacia* before fertilization, showing middle-piece.

B, sperm after entrance, showing middle-piece. Entrance-cone.

C, Entrance of sperm before rotation, showing entrance-cone. No middle-piece demonstrable.

D, sperm-star developing from base of sperm before rotation.

E, sperm rotated and advancing to egg-nucleus. Shows slight bulging of egg-nucleus toward sperm.

F, meeting of sperm and egg-nucleus. Sperm still conical; sperm-star still small; and projection of egg-nucleus to meet sperm.

pigmentation, and size, there are some minor points of divergence.

The sperm enters the egg at any point of the periphery, and an entrance cone, which soon disappears, is formed as it enters. The middle-piece, which can be distinguished before entrance (see Fig. 7, *A*), *may still be seen in favorable cases immediately after penetration* (see Fig. 7, *B*). Shortly after entrance the conical sperm-head rotates, but at times the aster develops at the base of the head before rotation, showing conclusively that it is derived from the base and not from the tip of the sperm-head (see Fig. 7, *D*). This aster remains much smaller than in *Toxopneustes*, and, in contrast to the latter, the sperm-nucleus retains its conical shape, generally, until it has met the egg-nucleus. No centrosome is to be found in the sperm-aster, or in any subsequent stage of the development of the latter. No radiations or centers are to be seen about the egg-nucleus before the sperm reaches it. Preceded by its aster and blunt-end first, the sperm advances to the egg-nucleus which puts out a process to meet it (see Fig. 7, *E* and *F*). The two nuclei meet in about five minutes; the sperm-nucleus quickly becomes vesicular, and later fuses completely with the egg-nucleus. The small sperm-star, which has meanwhile been forced to one side, now grows rapidly and gives rise to a large, granular, archoplasmic-mass, difficult of analysis, which passes down about the egg-nucleus (see Fig. 8, *A*). From the indefinite periphery of this mass, coarse rays, staining blue in iron haematoxylin and Congo red, run outward to the periphery of the egg. Gradually this mass draws together at opposite poles of the nucleus (see Fig. 8, *B*), the coarse rays disappear, and there are formed two small, dense red-staining archoplasms, surrounded by a blue halo of closely set, granular, fine astral rays. These form the archoplasms of the first segmentation-amphiaster (see Fig. 8, *C*).

The central masses of these asters from now on stain a bright vermilion in the double stain. They do not greatly increase in size, although the nucleus increases in bulk over eight times (see Fig. 8, *C*), until the separation of the chromosomes, when they grow rapidly and reach their maximum as

the chromosomes are farthest separated. Their structure may now be clearly seen. It is an irregular, granular reticulum, the meshes of which are filled with a matter which does not stain, and into the red reticulum the blue rays, spindle and aster, are directly continuous (see Fig. 8, *D*).

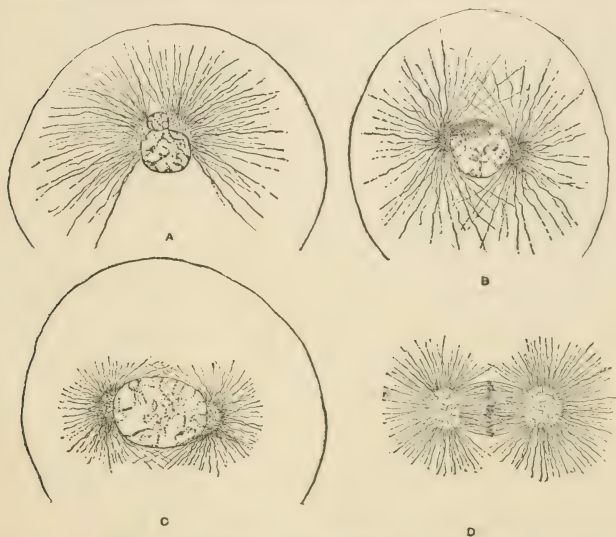


FIG. 8. *Arbacia*. *A*, growth of the archoplasm about the egg-nucleus. Development of the large rays, and the first appearance of division of archoplasm. Sperm-nucleus vesicular.

B, two nuclei fused, the sperm appearing as a dark cap at upper part of egg-nucleus. The archoplasm now completely divided. The nucleus has increased very little in size.

C, pause-stage. Coarse rays have disappeared. Nuclei completely fused. Illustrates great increase in size of nucleus at this time.

D, structure of the first cleavage-spindle, showing the reticulum in the archoplasms.

CONCLUSION.

The sperm-asters of *Asterias* and *Arbacia* have now been followed step by step, from their first beginning, throughout their growth, and directly to the asters of the first cleavage-spindle. There is no centrosome demonstrable about the

sperm, or in the first cleavage-asters, at any time, though the asters are large, perfectly distinct, and in the anaphase of the first cleavage the structure of the archoplasms may be seen with striking clearness. This failure to demonstrate centrosomes cannot be due to faulty method, for the centrosomes of the polar spindles have been recognized in their infancy, traced in their growth, and clearly followed to their respective ends.

In *Arbacia* there are at no time any radiations in the mature egg other than those proceeding from the sperm-archoplasm. In *Asterias* where the egg-centrosome and its archoplasm are to be seen, the latter may be followed in its steps of disintegration until it passes from view long before the union of the two nuclei. Finally Fol's picro-osmic mixture when employed upon *Arbacia* eggs, as in *Toxopneustes*, conclusively shows that the colorless aureole figured by Fol is an artefact caused in large part by the destruction of the archoplasm which should occupy this space. This aureole was readily obtained, yet within it, among the shreds and tatters of the archoplasmic-mass, no pictures of centrosomes resembling those of Fol could be obtained.

BIOLOGICAL LABORATORY OF COLUMBIA COLLEGE,
NEW YORK, December 11, 1894.

JOURNAL
OF
MORPHOLOGY.

EDITED BY

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WITH THE CO-OPERATION OF

EDWARD PHELPS ALLIS.

Milwaukee.

VOL. X.

FEBRUARY, 1895.

No. 2.

BOSTON, U.S.A.:
GINN & COMPANY.

AGENT FOR GREAT BRITAIN:
EDWARD ARNOLD,
*37, Bedford Street, Strand,
London, W.C.*

AGENTS FOR GERMANY:
R. FRIEDLÄNDER & SOHN,
*Berlin, N.W.,
Carlstrasse 11.*

AGENT FOR FRANCE:
JULES PEELMAN,
*189, Boulevard Saint-
Germain, Paris.*

CONTENTS OF No. 2, FEBRUARY, 1895.

| | PAGES |
|---|---------|
| I. <i>The Early Development of Amblystoma, with Observations on Some Other Vertebrates.</i> ALBERT C. EYCLESYMER | 343-418 |
| II. <i>The Formation of the Fish Embryo.</i> T. H. MORGAN | 419-472 |
| III. <i>On the Structure of Bimastos Palustris, a New Oligochaete.</i> H. F. MOORE : | 473-496 |
| IV. <i>The Anatomy of Bdellodrilus Illuminatus, an American Discodrilid.</i> J. PERCY MOORE . | 497-540 |

JOURNAL

OF

MORPHOLOGY.

THE EARLY DEVELOPMENT OF AMBLYSTOMA, WITH OBSERVATIONS ON SOME OTHER VER- TEBRATES.

ALBERT C. EYCLESHYMER.

TABLE OF CONTENTS.

| | PAGE |
|---|------|
| I. CLEAVAGE TO GASTRULATION | 346 |
| 1. METHODS | 346 |
| 2. THE UNSEGMENTED OVUM | 347 |
| 3. CLEAVAGE OF AMBLYSTOMA | 348 |
| 4. CLEAVAGE OF PETROMYZON..... | 355 |
| 5. CLEAVAGE OF COREGONUS..... | 357 |
| 6. GENERAL CONSIDERATIONS | 359 |
| II. GASTRULATION TO CLOSURE OF NEURAL FOLDS | 366 |
| 1. FORMATION AND CLOSURE OF THE BLASTOPORE IN AMBLY- STOMA | 366 |
| 2. ORIGIN OF THE MESOBLAST AND CHORDA | 370 |
| 3. EXPERIMENTS..... | 373 |
| 4. THE EMBRYONIC AREA | 375 |
| 5. THE PRIMITIVE STREAK AND GROOVE | 376 |
| 6. THE EPIBLAST AND MESOBLAST IN PETROMYZON..... | 378 |
| 7. GASTRULATION AND FATE OF THE GERM-RING IN AMIURUS AND LOPHIUS | 381 |

| | PAGE |
|---|------|
| 8. THE MESOBLAST IN FISHES | 384 |
| 9. GENERAL CONSIDERATIONS | 386 |
| 10. THE NEURAL FOLDS IN AMBLYSTOMA..... | 393 |
| 11. THE OPTIC VESICLES IN AMBLYSTOMA, NECTURUS AND RANA.. | 394 |
| 12. THE OLFATORY AND ORAL GROOVES IN RANA..... | 398 |
| 13. THE PARAPHYSIS AND EPIPHYSIS IN AMBLYSTOMA | 399 |

THE following work was begun at Clark University in the autumn of 1892, and continued during the succeeding summer in the Marine Biological Laboratory at Woods Holl. Since that time it has been carried on in the Zoölogical Laboratory of the University of Chicago.

An attempt to express my gratitude to Professor C. O. Whitman, at whose suggestion the study was undertaken, and whose criticism has ever been a source of inspiration, would indeed prove futile and sound extravagant.

The intention was at first to study certain phases in the ontogeny of *Amblystoma punctatum* (Baird), but the work has been gradually extended so as to include a comparison of particular features of development in *Necturus*, *Rana*, *Petromyzon*, *Amiurus*, *Lophius*, and *Coregonus*.

I am indebted to Professor C. O. Whitman for *Necturus* material, to Dr. W. M. Wheeler for *Rana palustris*, to Dr. H. P. Johnson for *Petromyzon*, to Mr. A. D. Mead for *Lophius*, and to Professor J. E. Reighard for *Amblystoma tigrinum*.

Habits, Oviposition, etc. — *Amblystoma* is probably the most widely distributed of all the Urodela, being found in nearly every state of the Union, as well as in Canada and Mexico. For its systematic position the reader is referred to the works of Cope, Hay, Garman, and others.

For observing the aquatic habits of these animals early spring is most favorable, since at this time they migrate to the ponds where the eggs are deposited. The time of their appearance varies in different localities. For the past four or five years, in the vicinity of Ann Arbor, Mich., they have appeared regularly on or about the 22d of March. At Worcester, Mass., I found eggs, presumably of *Amblystoma punctatum*, on April 6, 1892, while in eastern Wisconsin the animals were observed April 15, 1891.

The migration to the breeding grounds probably occurs at night, since they are rarely seen on land during the day. Their abrupt appearance in large numbers has often been noted, and at this time they may be easily captured; their appearance is no less sudden than their departure, — in two or three days they become extremely scarce and soon disappear.

They seem to be carnivorous; dissections of the alimentary tract indicate that their food is largely Crustacea. The young eagerly devour pieces of meat or insect larvae. In the absence of other food they prey upon each other.

The female is very choice in selecting a place to deposit her eggs, striving to obtain a position where she can rest upon a small twig or blade of grass and at the same time keep her nostrils above the water. She then clasps the twig between her posterior limbs and presses it tightly against the cloaca. The eggs escape singly, and adhere to the object on which they are deposited. The process may be continuous, covering a period of one or two hours, or at irregular intervals, lasting from a few hours to a day. The eggs may be deposited singly or in masses of a hundred or more.

In some localities the water is so turbid that considerable difficulty is experienced in obtaining the eggs. By placing a number of bushes in the pond the animals were attracted; the following morning the bushes were raised and a part of the eggs collected. In this way freshly deposited eggs were readily obtained.

The fresh eggs are so closely crowded together and firmly held in place by the gelatinous envelopes that they can only be removed with difficulty; after remaining in the water for a few hours the envelopes swell and the removal is easily effected. At this time the egg presents the appearance indicated in Pl. XVIII, Fig. 1, being enclosed by three concentric envelopes. The outermost, by means of which the eggs become attached to the object on which they are deposited, is transparent and structureless. Numbers of minute algae are often found in this membrane; but their presence in the internal envelope, as noted by Orr ('88), has not been observed. The middle envelope presents a more irregular outline. When

treated with Schneider's acetic carmine a finely punctated appearance is revealed, probably due to the presence of minute pores. The inner or vitelline membrane is always so closely applied to the egg that it is not easily detected.

I. CLEAVAGE TO GASTRULATION.

I. METHODS.

To plainly observe the phases of cleavage it is necessary to remove the envelopes. This is best accomplished by seizing the mass with forceps and at the same time piercing and snipping the envelopes with spring-scissors, allowing the eggs to fall to the bottom of the dish. It is often desirable to fix the eggs before the laboratory can be reached. I found it a good plan to carry a jar of Perényi's fluid and to place the eggs at once in this; when the laboratory is reached the envelopes may be removed, or the mass transferred to 70% alcohol and preserved indefinitely. When ready for study they are passed through 50% alcohol to water, and the envelopes removed by using a weak solution of hypochlorite of sodium (eau de Labarraque).

In the study of the living egg a plane mirror was placed beneath the watch-glass containing the egg as suggested by Pflüger ('83). This is of great assistance, since the changes going on at either pole may be observed without disturbing the position of the egg.

Various reagents have been employed in fixing, among these Perényi's fluid stands first for general histology. Picro-acetic gave excellent results, especially in cleavage. Chrom-formic and chrom-osmo-acetic were also used.

Among the many stains used, I especially recommend Czokor's alum-cochineal, Mayer's alcoholic-carmine, and Grübler's borax-carmine. I have used the Biondi-Ehrlich mixture, also borax-carmine and nigrosine for double staining with excellent results. The staining has been tried both *in toto* and in section, the latter invariably giving the better differentiation.

In imbedding both the celloidin and paraffine methods have been employed. Owing to the crumbling of the yolk and the

shrinkage of the eggs, often producing artificial delaminations and cavities, it was found necessary to discard the latter. Serial sections by the method which I have described in another place ('92) have been used for the most part. A method which I found very instructive was to imbed several eggs without reference to planes. This obviously necessitated reconstructions.

In reconstruction the wax plate method gave most satisfactory results. I have also profited by camera lucida drawings on panes of glass; the outlines of the various structures being drawn in water colors. By combining these, a graphic representation of the embryo is obtained. Projections on ruled paper have also assisted in gaining an idea of the structures.

In the present paper I shall omit entirely the discussion of the phenomena of maturation and fertilization, since these subjects have been so thoroughly studied by Jordan ('93) in the Newt and Fick ('93) in the Axolotl.

2. THE UNSEGMENTED OVUM.

The fertilized ovum of *Amblystoma* is nearly spherical, being slightly flattened at the pigmented pole and measuring about 2 mm. in diameter. The upper portion presents a deep brown color (Pl. XVIII, Fig. 1), which in a zone just above the equator changes quite abruptly to a lighter shade, and fades away into a faint yellow at the opposite pole. A marked difference in the density of pigment on the opposite side of the upper hemisphere is often noticeable. While this difference is by no means constant, I believe it of sufficient importance to be kept in mind with reference to the orientation of the embryo.

If the egg be examined a few hours after deposit it presents the appearance shown in Fig. 2. There is a very conspicuous saucer-shaped depression surrounded by a zone in which the pigment is less dense than in other portions; to this area various names have been applied, as, "Keimpunkt," "fovea germinativa," "troch vitellin," "light spot," *etc.* Slight magnification reveals at its center a small pit in which the first polar globule lies.

The superior pole some hours later (Fig. 3) shows no trace of the saucer-shaped depression. The pit at its center has elongated as indicated in Figs. 3 and 4. In the meantime a second polar body has appeared. The elongation or slit-like extension of the pit was also observed in the eggs of *Rana*.

I have sectioned a number of eggs at this stage to determine what relation, if any, the pigmented path of the spermatozoön bears to this slit, but no fixed relation has been discovered.

In eggs where the pigment is eccentric I have likewise endeavored to determine what relation a line drawn through the centers of the lightest and darkest portions bore to this slit, but efforts in this direction were also fruitless.

3. CLEAVAGE OF AMBLYSTOMA.¹

In the following pages I shall describe the successive stages of a particular egg which presents a fairly typical cleavage, recording in each case the variations most frequently observed in the twenty to thirty living eggs studied; these variations being confirmed by a study of hardened material.

The term cleavage, as here used, covers the whole period of cell-formation up to the time of gastrulation.

First cleavage. — The appearance of the first furrow is foreshadowed by a flattening at the superior pole, which soon changes to a furrow. The progress of this furrow over the dark hemisphere is rapid and continuous, covering a period of less than 15 min. As the furrow passes toward the opposite pole its rate of progress constantly decreases, and it is 1 hr. and 53 min. (Fig. 13) before the entire periphery of the egg is encircled by a continuous furrow (Fig. 5). The furrow is vertical, dividing the egg, as a rule, into two nearly equal parts. Although I have often observed the progress of the ends of the furrow, I have never found any indication of an alternate widening and narrowing of the opposite ends as recorded by von Baer ('34).

An interesting phenomenon which occurs during the progress of the furrows is the formation of the so-called "Faltenkränzen." Whether they are to be regarded with Reichert ('41) and others

¹ Some of the facts presented in this portion of the paper are the results of a joint study with Dr. E. O. Jordan ('94). I gladly acknowledge my indebtedness to him.

as a consequence of the contraction of the yolk, or are to be considered with Max Schultze ('63) as an expression of a radial arrangement of the yolk particles, I am unable to determine.

The origin of the furrow with reference to the slit-like orifice already described has been carefully studied. Unless special attention be directed to this, one is very apt to infer that it is the beginning of the first cleavage. More careful study shows the two are entirely independent, and that no fixed relation exists between them. In Petromyzon, Calberla ('78) believed the two to coincide.

It is by no means always the case that the first furrow divides the egg into two equal parts; it may depart so far from a meridional that the segments formed are quite unequal, as observed in the Frog by Prévost and Dumas ('24), Rusconi ('36), Newport ('51), and others. Neither do the ends of the furrow always unite with each other in a straight line, but are often joined in such a manner that an obtuse angle is formed.

Sections of this stage show that the yolk is highly differentiated, the granules at the vegetative pole being largest, and gradually decreasing in size toward the animal pole until the upper portion is constituted of a finely granular protoplasm.

Second cleavage. — In many cases before the ends of the first furrow have reached the inferior pole, the appearance of a second set is foretold by the rapid closing of the groove at the dark pole; this continues until there is but a faint indication of its presence.

The second furrows begin at the first, and extend in either direction at right angles to it (Fig. 6). Their progress is in all essentials like that of the first, and in 1 hr. and 46 min. after the time of appearance they have reached the opposite pole.

Variations in the formation of the second set of furrows are not uncommon. The point of origin may be at the pole or at a greater or less distance from it. Newport ('51) observed this eccentric origin, and believed that the second furrows formed segments which on one side were always larger than those on the other.

Instead of a right angle, acute and obtuse angles may arise in such a manner that they form an X, as figured by Prévost

and Dumas ('24). Again, the furrows may not arise in the same locality, in which case the two are united by the short piece of the first furrow intervening, to which the designation "cross-furrow" has been applied.

These furrows do not always begin at the same time; one may precede the other by a considerable interval. At the vegetative pole many variations are likewise found; often the two join to form a continuous line running at right angles to the first, again forming acute and obtuse angles.

In the axial line where the four quadrants of the egg join, along a region just above the center of the egg, sections show an open space, which is the first indication of the segmentation cavity.

Third cleavage.—In 1 hr. 45 min. after the appearance of the second set of furrows, and even before they have reached their destination, there are indications of the first horizontal division at or near the limits of the dark hemisphere (Fig. 7). In the particular egg from which the diagrams are made, quadrant *b* (Fig. 14) was the first to divide, the furrow started from the second vertical (indicated in the figure by a dotted line) and progressed from left to right. In 4 min. it reached the first vertical.

The next division occurred in the adjacent cell *d* a few moments later, extended in the same direction, and reached its destination in about the same time; 3 min. later a third furrow, starting from the first vertical, appeared in cell *a*, and progressed in the same direction as the two preceding. Its rate was more rapid, however, reaching the point at which the first terminated in less than 2 min. Before this furrow was complete, cell *c* divided from right to left. The entire time occupied in the formation of this set of furrows is extremely brief as compared with the preceding cleavage.

In point of origin and direction of progress many variations occur. They may depart from a common point at either the first or second vertical. In many cases they appear successively in the different quadrants, following the direction of the hands of a clock, again passing in precisely the opposite direction.

It often happens that some of the furrows, instead of being horizontal, are more or less inclined, at times passing into the vertical. Jordan and myself ('92) described such variations in the Newt, Frog, and Amblystoma. Morgan and Tsuda ('94) have since recorded similar variations in the Frog's egg.

Fourth cleavage. — About 1 hr. and 50 min. later, the fourth set of furrows appear. In the majority of cases they are vertical, as shown in Fig. 8. They often all depart from one or the other of the first two verticals; sometimes, however, some depart from one, while the rest depart from the other, as shown in Fig. 15. In short, this set offers so many variations that a type is no longer recognizable. In the egg represented (Fig. 15) the first of this set appeared in quadrant *b* at the first vertical. The next was formed in the adjacent quadrant *a* 2 min. later, its point of origin being at one of the second verticals. The third furrow, starting from the first vertical, passed across quadrant *d* 1 min. later; the division of *c* followed 1 min. later, and this set passed toward the lower pole, which point some reached, while others turned aside and united to form a common furrow.

The entire set of verticals may depart from a common point at the superior pole and cut each quadrant into two approximately equal portions, in which case a radially symmetrical condition obtains. It was this condition which von Baer ('34) observed in *Rana* and considered as a type. Yet Rauber ('83) denies this a typical value, and states that he has not seen a single egg where such was the case.

Another condition often observed is one in which the position of the furrows in hemisphere *ab* (Fig. 15) would be similar to that in *cd*; in this case there is a distinctly bilateral appearance. The furrows often run nearly parallel to the first vertical, and again parallel to the second, recalling the condition observed in Teleosts. Such variations in the Frog's egg have been figured by Prévost and Dumas ('24), Max Schultze ('63), Rauber ('83), and others.

Fifth cleavage. — In this cleavage (Fig. 9) the furrows are both horizontal and vertical, the majority being horizontal. If we follow this cleavage in detail (Fig. 16), we find the first fur-

row appearing in quadrant *d*, passing from the first to the third vertical in a horizontal direction. The next furrow appeared in quadrant *a* 2 min. later, extended in the direction intermediate between horizontal and vertical, and terminated in the second vertical; 1 min. later a horizontal passed from the second to the third vertical in quadrant *c*. In a few moments horizontals appeared simultaneously in the adjoining cells of quadrants *b* and *d*. A horizontal 1 min. later passes between the third and first verticals in quadrant *a*; 1 min. later verticals depart from the first vertical in quadrants *b* and *c*.

Although I have never found this cleavage entirely vertical, it has been observed in the Frog by Prévost and Dumas ('24). They say: "Au même instant deux nouveaux sillons parallèles à celui qui s'était montré le second sur la partie brune, viennent se dessiner sur elle d'abord sous la forme d'une trace légère, et bientôt ils atteignent une profondeur analogue à celle de leurs prédécesseurs. Cet hémisphère se trouve alors divisé en seize parties égales ou à peu près."

Later cleavage.—The following cleavage (Fig. 10) is likewise made up of both verticals and horizontals. The majority are generally vertical, occasionally horizontal. The corresponding diagram (Fig. 17) illustrates the times of the divisions. The interval from the beginning of the first to the last furrow is 15 min. A vertical section through the lighter and darker regions of such an egg is shown in Fig. 20. The cells forming the roof of the segmentation cavity (*sc*) vary in size and pigmentation, the larger being more deeply pigmented.

In the next cleavage (Fig. 11), as in the preceding, the furrows pass in both horizontal and vertical planes. In the corresponding diagram (Fig. 18) the times are but partially recorded; it being at this time extremely difficult to observe the cleavage over the entire surface. The interval between the first and last furrows is 20 min.

The differences in color observed in earlier stages have gradually become more pronounced, so that at present in nearly all eggs differences exist. The lighter portion fades out gradually in the region of the equator, while the darker portion has a more distinct line of demarcation. In many eggs the surface

views indicate an acceleration of cleavage in the least pigmented portion, yet sections (Fig. 21) show the region possessing most pigment to be that in which cell activity is greatest.

After the cleavage shown in Fig. 12 it is impossible to follow the divisions, even in areas ; but by magnifying the egg some 30 diameters individual cells may be observed. At this time the cleavage of any particular cell occurs at intervals varying from 1 hr. to 1 hr. and 45 min.

Fig. 22 represents a vertical section through the more and less deeply pigmented portions. In the more deeply pigmented portion the roof of the segmentation cavity is several layers of cells thick, while in the less deeply pigmented portion it has remained a single layer. The relation which the more deeply pigmented portion bears to the blastopore and to the future embryo will be discussed presently.

Experiments on cleavage. — Hertwig's ('93) recent experiments on the Frog and Triton, in which the second furrow was made to pass in a horizontal direction, and in which the path of the succeeding furrows bore a definite relation to the direction and degree of compression exerted, indicate that the planes of cleavage may be entirely changed through the effects of mechanical pressure.

Experiments of the same kind were made on the eggs of *Amblystoma tigrinum* in April, 1893, in the Morphological Laboratory of the University of Michigan. Unsegmented eggs, from which the envelopes had been removed, were placed between the cut edges of two glass slides and laterally compressed to one-half their equatorial diameter. In order to observe both poles of the egg, the experiments were carried on in glass dishes placed upon a mirror.

The first vertical in the 34 eggs examined showed no constant relation to the compressed surfaces, in 7 passing through the longest equatorial diameter ; in 9, through the shortest ; and in 18, between the two. The second verticals showed no greater variation than under normal conditions. The following cleavage was generally equatorial, yet in some cases, 3 out of 34, these furrows were vertical. Beyond this cleavage many

irregularities were observed, no greater, however, than occur in normal cleavage.

Some of the compressed eggs died before the neural folds were formed. A number, however, produced normal embryos, and the longitudinal axis, when compared with the drawings made of the first and second cleavage furrows, showed in the 15 eggs examined variations ranging from 5 degrees to 45 degrees.

As a result of pricking the egg, a minute quantity of protoplasm was extruded forming extra-ovates which remained attached to the surface of the egg until the embryos were well developed. In a number of cases eggs in the two-cell stage were pricked on either side of the first furrow. I more frequently found the extra-ovates on the same side of the median line of the embryo, but occasionally on opposite sides.

The extra-ovates when detached exhibit interesting phenomena, segmenting for a time synchronously with the egg; the cells arrange themselves so that lighter and darker hemispheres are formed. In short, the extra-ovate is a miniature egg. I have not been able to rear any of them beyond the blastula.

In 5 other experiments a delicate silk thread was tied around the egg in the first and second cleavage furrows. The results were likewise variable; in 3 eggs the long axis of the embryo formed right angles with the thread, in 2 oblique angles were formed.

While these experiments indicate that no constant relation exists between the axes of the embryo and cleavage furrows, they are open to the objection that abnormally compressed eggs may reveal nothing of the normal relations.

Rotation of ovum. — The continuous rotatory movement of the vertebrate ovum so often observed by the older naturalists; Leeuwenhoek, Carus, Sharpey, Bischoff, and others, is a phenomenon as yet unexplained. Sharpey, Rusconi, and Bischoff held this to be due to the presence of cilia, yet Barry, Aubert ('54), and others failed to detect their presence, the latter attributing the movement to osmotic action.

In Amphibia (Triton, Amblystoma, Rana) the rotation of the egg within the membranes is most noticeable during the

period between the late blastula and the closure of the neural folds. I have often observed this movement going on in a large number of eggs at the same time, some rotating in one direction, others in the opposite.

Clarke states that in *Amblystoma punctatum* the surface of the body is covered with cilia, at the time the neural folds close, by means of which it keeps up its rotary motion. I have endeavored to detect cilia by teasing in normal saline solution, also by osmic acid fixation, but without success.

4. CLEAVAGE OF PETROMYZON.

In the study of this form I have been greatly assisted through the kindness of Dr. Johnson, who has not only placed his material at my disposal, but also has allowed me to use some of his notes and drawings on cleavage.

First cleavage. — The first furrow (Pl. XIX, Fig. 1) appears at the superior pole in from 4 to 5 hours after fertilization, the ends may progress at a uniform rate or one may exceed the other in rapidity. The furrow is completed 15 to 30 min. later. In the majority of cases the cells are nearly equal, but exceptions occur; out of 57 eggs examined from different lots of material 12 showed quite decided variations. One of these variations is shown in Fig. 5.

So far as I am aware, the only observation showing a marked difference in the formation of the first furrow is that of Calberla ('73), who found the first cleavage in a horizontal plane, dividing the egg into two unequal parts. As this condition has never since been observed, it is fair to suppose that it occurs but rarely.

Second cleavage. — The formation of the second furrow begins in about 1 hr. 30 min. after the appearance of the first, progresses in a manner quite similar, and forms with it right angles. In most eggs a cross furrow is present; it may arise as in *Amblystoma* by the two furrows starting from widely separated points, or may be the result of a shifting of the blastomeres as described in *Amphioxus* by E. B. Wilson.

One furrow of the second set may extend at right angles to the first, while the other forms an acute or obtuse angle, or both may form acute and obtuse angles as shown in Figs. 10 and 11. Both furrows of the second set may fall far to one side of the upper pole, forming two larger and two smaller cells (Fig. 9). One of the furrows may reach the opposite pole before the other has started, or it may not appear at all as a vertical but as an equatorial; such a case was observed in the living eggs shown in Figs. 5 and 6.

Third cleavage. — The third cleavage occurs in about 1 hr. and 40 min. after the second. This set is often horizontal, occasionally vertical, again a part vertical, a part horizontal, and others which fall between the two so that it becomes impossible to select a type. A few of the more frequent variations are recorded.

A variation is shown in Fig. 10, where three blastomeres are cut off by horizontal furrows, while in the remaining quadrant no furrow is yet formed, although one of the fourth set (4) has appeared.

In Fig. 7, horizontals (3) are present in two quadrants only, giving rise to two smaller micromeres; while a third furrow (3) passes vertically, taking a direction and position which would permit its interpretation as one of the second set. Again, in the egg shown in Figs. 3 and 4, three quadrants are divided by verticals, while in the fourth a "tangential" (Kupffer, '90) is formed. In Figs. 12 and 13 we observe a set of verticals, and an entire absence of horizontals.

The variations recorded may offer a solution to what has hitherto given rise to no little perplexity concerning this cleavage. The majority of observers maintaining that this cleavage is horizontal while McClure ('93) holds the typical method to be the formation of a set of verticals followed by horizontals.

The succeeding cleavage is represented in Figs. 14 and 15. The directions of the furrows are so varied that their classification is impossible, as is evident not only from my own observation but also from the fact that scarcely two observers agree as to its nature. Max Schultze ('56) held that two more equatorials follow the third. Shipley ('88) states that it is followed

by two meridionals. According to Kupffer ('90) either may occur and from the third cleavage on no regularity exists. Kupffer also adds a third order which is called tangential. Comparing the variations in the cleavage of *Amphibia* and *Petromyzon* we observe in the second and third acts of the latter the irregularities manifested by the third and fourth of the former.

5. CLEAVAGE OF COREGONUS.

The observations were made largely on preserved material, yet notes made three years ago on the cleavage of the living egg have served as a guide.

First cleavage. — The first furrow may divide the blastodisc into two equal parts as in Pl. XIX, Fig. 16, where they lie at first closely together, but soon assume the condition indicated in Fig. 17, in which they lie quite apart; or they may later become widely separated at either end as in Fig. 18. In case the first furrow gives rise to two unequal blastomeres, which is the usual occurrence, it is impossible to classify the variations, since they range from almost equal size to a condition in which one is two or three times the size of the other, as in Fig. 19.

This inequality in size of the first two blastomeres is one often observed in Teleosts. Rauber ('83) observed it in *Gobius*, Agassiz and Whitman ('84) in *Ctenolabrus*, Henneguy ('88) in *Salmo*, and H. V. Wilson ('91) in *Gadus*, yet all produced normal embryos.

Second cleavage. — The second set of furrows may form right angles with the first, giving rise to four equal blastomeres (Fig. 22). They may pass from the same point at one side of the pole, resulting in two smaller and two larger blastomeres (Fig. 24). Again the two furrows may depart from points widely separated (Fig. 23) as in *Amblystoma* and *Petromyzon*. If two unequal blastomeres are produced by the first cleavage, a variation often occurs in which the smaller blastomere remains undivided (Figs. 20 and 21) during this cleavage. Again the larger may divide twice while the smaller undergoes but a single division, as in Fig. 25, or the opposite may occur (Fig. 26), although seldom observed.

In the eggs of *Merlucius*, studied by Kingsley and Conn ('83), "the blastomeres varied widely in size, and the segmentation furrows progressed at varying rates in different portions of the germinal areas." Agassiz and Whitman ('84), Henneguy ('88), and others have observed like differences in the size of the blastomeres of the second cleavage. I have never found the second furrows passing in a horizontal plane, yet Kupffer ('68) found this to be the case in the Herring, and List ('87) observed the same in *Crenilabrus*.

The condition represented in Fig. 27, presumably derived from a form like that shown in Fig. 23, is of rare occurrence.

The form depicted in Fig. 28 is one likewise seldom observed, and it may be said that in the White-fish the typical six-cell stage of other Teleosts is only one of the many variations.

The meridional nature of the third cleavage in Teleosts is generally agreed upon, with the exception of Brook ('86) who holds that it is equatorial.

In the eight-cell stage, if such it may be considered, the cells show endless variations in arrangement, the bilaterally symmetrical form shown in Fig. 33 being very seldom found, that indicated in Fig. 32 more often, and most frequently some variation of that observed in Fig. 30. A radial arrangement about a central cell (Fig. 29) is occasionally observed.

While the majority of investigators have considered the cleavage of the Teleost-ovum as fairly regular, we find the cleavage of *Coregonus* quite the opposite; we may also infer from the work of Rauber ('83) that in certain forms if any two blastoderms, in late cleavage, were superimposed the furrows would nowhere coincide. Ryder also states "that it is probably true that most eggs will be found to vary more or less notably."

The forms shown in Figs. 30, 31, and 32 are probably the derivatives of an unequal two-cell stage. The wide separation of these groups of cells is a point of interest. A like condition has been figured by Miss Clapp ('91) in the eight-cell stage of the Toad-fish, but nothing is said concerning its significance.

There often exists the peculiar condition indicated in Fig. 35 in which there is a marked separation of the derivatives of the early blastomeres. This at once suggests the beginnings of double embryos; while this seems very plausible, it is nevertheless improbable, since double embryos are but rarely observed.

Brauer ('94) has observed the same separation in the egg of the Scorpion, yet has not been able to determine whether the parts later unite or remain separated and form double embryos.

Another curious fact is the existence of extra-ovates which keep pace with the segmentation of the blastodisc and show no connection with it whatever. They appear to originate from the layer of cells extending over the yolk, which was presumably in an earlier phylogenetic stage covered by the blastodisc.

6. GENERAL CONSIDERATIONS.

Polarity.—One of the most striking features of the Amphibian ovum is its tendency to orient in such a manner that the darker hemisphere is uppermost. Von Baer in 1834 called attention to this difference between the upper and lower hemispheres of the egg, and by it determined the primary axis of the ovum. The distribution of pigment, the formation of polar globules, the initiation of cleavage, and the accompanying "Faltenkränzen" serve further to emphasize this polar differentiation.

Among those who first called attention to this phenomenon may be mentioned Auerbach, Balfour, and Lankester.

Hatschek ('77) states: "Es ist wahrscheinlich, dass eine polare Differenzirung schon an der ungefurchten Eizelle bei allen Metazoen statt hat, durch welche der vegetative und animale Keimpol bestimmt ist."

Whitman ('78) observed a marked polarity in the egg of *Clepsine*, and emphasized its importance.

Hallez ('85) has noted its occurrence in insect ova, and says: "La cellule-œuf possède la même orientation que l'organisme maternel qui l'a produite; elle a un pôle céphalique et un pôle caudal, un côté droit et un côté gauche, une face dorsale et une face ventrale."

Van Beneden ('83) observed this to be true not only in the unsegmented but also in the unfecundated egg of *Ascaris*. "Un examen attentif de la forme extérieure de l'œuf, et plus encore l'étude de sa structure, démontrent l'existence dans ces œufs d'un axe morphologique, dont les extrémités, que j'appelle les pôles de l'œuf, présentent une valeur tout différente tant au point de vue anatomique qu'au point de vue physiologique. L'un de ces pôles est prédestiné à recevoir le zoösperme : par ce point seul l'élément fécondateur peut pénétrer dans le vitellus."

One is accordingly forced to believe that we must assume a polarity not only in the unsegmented egg, but at a much earlier period, even preceding fecundation.

Mark ('90) has shown that in *Lepidosteus* the ovarian egg early exhibits a polar differentiation, indicated by a secretory activity of the protoplasm at a point which corresponds to the future micropyle.

In this connection the observations of Stauffacher ('93) on the eggs of *Cyclas* are of interest. There is a certain area, over which the egg membrane does not extend, which later becomes the micropyle. Stauffacher found this area to be the point by which the early egg-cell is attached to the follicular wall; one pole of the egg is thus pre-determined in the germinal epithelium.

One of the most noticeable features in the central nervous system of the larva of *Amblystoma* is the polarity of the nuclei indicated by the peculiar eccentric position of the chromatin. Mall ('93) likewise noted its presence in the cells of the retina of *Amblystoma*, and considered its importance in the development of the nerve fibre.

Rabl ('89) has called attention to a polarity existing in the nuclei of the epithelial cells of the Salamander.

In the nuclei of the intestinal gland cells of *Ptycoptera*, Van Gehuchten ('89) has observed this polarity and emphasized its importance.

The above facts not only indicate that the ovic axis is established at a very early period, but also that this is one of the most fundamental characters of animal cells; and we may con-

clude with Hallez ('86) that "chaque élément histologique possède, lui aussi, ces deux polarités de l'animal, polarités qui persisteraient dans la cellule-œuf, après qu'elle a cessé de faire partie des tissus maternels."

Relation of ovic axis and embryo to cleavage planes. — One of the first questions to be considered is the relation of the first furrow to the ovic axis, the upper pole of which is marked by the point of exit of the polar bodies.

Schultze ('87) holds that the plane of the first furrow is determined by the ovic axis and the eccentric germinal vesicle, and that its direction may be anticipated in the unsegmented egg.

Roux ('88), on the other hand, maintains that the first cleavage is determined by the ovic axis and the path of the male pronucleus, as first stated by Newport in 1853.

In *Amblystoma*, *Petromyzon*, and *Coregonus* this furrow does not always pass through the ovic axis, nor does it bear a constant relation to it, except that it generally passes in a plane parallel.

This peculiarity Rauber ('83) has noted, and suggests that there must be a "Polflucht" through the influence of which the furrows are thrown away. Whether this be accepted or not the fact remains, and is of great importance, that this eccentricity commonly occurs, hence the ovic axis cannot be considered as a rigidly determining factor.

Concerning the relation of the succeeding furrows to the first, it seems conclusively shown from the variations recorded in the preceding pages, as well as the observations of Rauber ('83) and Jordan and myself ('94), that in the forms studied no fixed relation exists.

Another theoretically important question is the relation of cleavage furrows to the axes of the embryo.

Newport, in 1853, called attention to this in the following words: "I have long been aware that the axis of the embryo was in line of the first cleft of the yolk." In a following paragraph, however, the author states: "The observations point out that at times the axis deviates to the right or left of the line."

From a number of experiments Roux, in 1883, reached the following conclusion: "Mit der Ebene der ersten Furchung

wird beim Froschei zugleich auch die künftige Medianebene des Individuums bestimmt, und zwar fallen beide zusammen." From the facts recorded by Roux (pp. 14-16) it is evident that this coincidence was by no means constant.

In the same year Pflüger ('83) reached the conclusion that the first furrow represented the median line of the embryo. Supported by these eminent investigators, the idea received very wide acceptance. Rauber ('86) was one of the first to question the observations, and showed that in the Frog and Axolotl this was not true, but that the second meridional instead represented the median plane of the embryo. By consulting Rauber's tables it will be observed, however, that the embryo was formed at almost any angle to the first cleavage. The later experiments of Hertwig on the egg of Triton, in which a silk thread was tied through the first furrow, confirmed the observations of Rauber, as do also the observations of Jordan on the Newt.

The work of Miss Clapp on so favorable an egg as that of *Batrachus* seems to show that in this form, at least, the first plane of cleavage bears no definite relation to the median plane of the future embryo.

In 1892 Roux modified the position which he held in 1883, and states that "Bei den bilateralen-symmetrischen Tieren entspricht eine der beiden ersten Teilungsebenen der Medianebene des Embryo resp. des erwachsenen Tieres."

In case the second furrow coincides with the median plane, it is interpreted as being intrinsically the first. Roux says: "Es entsteht dann die normalerweise zweite, kopf- und schwanzwärts scheidende Furche als erste, und die normale erste, der Medianebene entsprechende Teilungsebene als zweite."

To my mind a serious objection to all theories postulating the coincidence of any cleavage furrow with the median plane of the embryo in *Rana*, *Bufo*, *Amblystoma*, and *Diemyctylus* is the fact, emphasized by Jordan and myself ('94), that the furrows undergo a decided torsion. A glance at Pl. XVIII, Figs. 13-18, will illustrate the point. This shifting of the cells, although often observed, has hitherto received but little attention. It was witnessed by von Baer in 1834, and described

as follows: "Es ist ein wunderbares Schauspiel, unter der Loupe diesen plötzlichen Tumult in Dotter Klümpchen zu sehen. Manches Individuum wird von seinen unruhigen Nachbarn eingemal hin und her geschoben bevor es zu Ruhe kommt."

Newport ('51) observed the same, and says: "In some ova there is such an unusual displacement of the segments as almost to prevent the identification of the parts in the subsequent changes." It thus becomes impossible for any furrow to coincide with a straight line forming the median plane of the embryo, unless after the egg has reached a very late stage, the cells shift back and arrange themselves along definite lines, which seems extremely improbable.

I believe the only rational conclusion which can be drawn is that no cleavage furrow in these vertebrates bears a fixed relation to the future median plane of the embryo.

Homology of cleavage furrows.—The supposed homology of the first cleavage furrows throughout the vertebrata is based upon its relation either to the ovic axis or the embryo. Since it bears no constant relation to either, it seems questionable if any homology can be drawn.

In attempting to homologize the second furrow more serious difficulties are encountered, since we must consider this cleavage as made up of two entirely independent cell divisions, the plane of which bears no fixed relations to the first furrow, either in time or direction, or to the ovic axis. If we compare the second meridionals in the two eggs of *Petromyzon* (Pl. XIX, Figs. 6 and 8), the difficulties are evident.

In comparing the Teleostean and Amphibian types of cleavage, Rauber was forced to postulate the loss of the first set of equatorials in the Teleost. H. V. Wilson believes such a loss probable and holds with Rauber that the equatorials in the Teleost are represented by the edge of the blastodisc.

Agassiz and Whitman think this improbable and accept the homology of the third cleavage in the two groups (according to Rauber and Wilson the third Amphibian and the fourth Teleostean).

It is evident that I cannot accept either of the above homologies and do not believe that even in the Amphibia we can

The gradual obliteration of the distinct periods of rest, and the consequent transition to a condition of constant activity is brought about at an early stage. It is of interest when compared with the regularity manifested in the cleavage of certain Teleosts (Serranus, Wilson, '91).

The transition is due to the early appearance of areas of special activity. While the region of greatest activity is undoubtedly at the superior pole, there is another portion of the blastoderm in which cell division becomes accelerated; this is that portion which is most deeply pigmented and which is destined to lie somewhere in the future tail region of the embryo.

Kölliker ('79) observed these differences in the embryonic area of the chick. I quote his words: "Die Furchung geht immer asymmetrisch vor sich, so dass ohne Ausnahme die eine Hälfte der Keimscheibe in der Zerklüftung der andern voran ist . . . der schneller sich furchende Theil zum späteren hinteren Theil des Blastoderma sich gestaltet, in dem die ersten Spuren des Embryo entstehen."

In the blastoderm of *Amphioxus* Lwoff ('92) describes an area of accelerated cleavage at the dorsal lip of the blastopore: "die Zellenvermehrung nicht überall gleichmässig vor sich geht, sondern sich vorzugsweise an einer Seite konzentriert, die zur Dorsalseite der Gastrula wird."

The fact that areas of accelerated activity appear in early cleavage stages is, as we shall see, one of fundamental importance in orienting the embryo.

If we compare the variations observed in the three types studied, it is evident that the greatest variation is in the Teleost while the least is in *Amblystoma* and *Rana*. The order of variation might be expressed thus: the first and second furrows in *Coregonus* show the irregularities found in the second and third of *Petromyzon*, while the second and third of *Petromyzon* show about the same degree of variation as the third and fourth of *Amblystoma*.

From the preceding pages we conclude that in *Petromyzon*, *Amphibia*, and Teleost the more detailed the study of cleavage the more apparent become the irregularities, and we wonder

that from eggs manifesting such diversities of cleavage identical embryos, so far as we are able to determine, arise. If abnormal embryos resulted, the variations from a given type might bear a direct causal relation; but from the evidence bearing upon this point such does not appear to be the case, and my study only emphasizes the truth of the statement made by Jordan and myself ('91) that "the irregularities of cleavage have no appreciable effect upon any stage of development of the embryo."

The above being true, two alternatives present themselves. Either the egg is isotropic, or its mosaic character is not revealed by its mode of cleavage. If the former be true, we are wholly unable to explain the many facts of precocious differentiation so obvious in the Annelids, Molluscs, and other invertebrates. To accept the latter is to admit the inadequacy of the mosaic theory as based upon cleavage phenomena.

II. GASTRULATION TO CLOSURE OF NEURAL FOLDS.

I. FORMATION AND CLOSURE OF THE BLASTOPORE IN AMBLYSTOMA.

About 60 hrs. after the first cleavage gastrulation begins. In order to follow the process in detail the following methods were employed: The egg, while still within its membranes, was placed upon a glass slide, and after allowing the membranes to dry just enough to hold the egg firmly *in situ*, the slide was inverted and placed over a moist chamber. This method together with the assistance of a bed of cotton upon which the eggs, with envelopes removed, could be easily held in position, have enabled me to observe the movement of individual cells.

At the close of cleavage there is an irregular broken line (*b.p.*, Pl. XX, Fig. 1) lying just below the equator and formed by the union of a number of cleavage furrows; along this line the process first begins. While in *Amblystoma* invagination always begins nearer the equator than the vegetative pole, other conditions have been observed in *Amphibia*. In *Rana* Pflüger ('83) and Roux ('88a) find the blastopore first appearing at the equator of the egg, while Houssay ('90) in the *Axolotl*,

and Jordan ('93) in the Newt, state that it first appears at the vegetative pole.

I have examined a large number of eggs at this stage to determine what relation this line bears to the more deeply pigmented area of the superior hemisphere to which I have already referred. In all eggs where the differences are sufficiently marked to admit of orientation, the irregular line lies beneath the darker portion and is parallel with its more sharply defined border. It will be recalled that the deeply pigmented area is the area in which cell-division has become much accelerated (Pl. XVIII, Figs. 21, 22).

Concerning this peculiar distribution of pigment there are but few references in the literature. Roux ('83) observed certain differences in the Frog as expressed in the following words: "Von der Gastrula ist es bekannt, dass schon bald nach Beginn ihrer Bildung die Rückenseite des Blastoporus an dunklerer Färbung kenntlich ist, womit zugleich der Punkt bezeichnet ist, an dem die Rückenfurche sich zu entwickeln beginnt."

Hertwig ('82) found in Triton "dass der Dotterpfropf keinen gleichmässigen Anblick darbietet, insofern eine Hälfte ganz pigmentfrei ist und weissgelb aussieht, die andere aber ein wenig bräunlich pigmentirt ist. Ferner ist auch der an die weissgelbe Hälfte des Dotterpfropfes angrenzende Theil des Eies viel schwärzer pigmentirt als die Umgebung der anderen Hälfte. Nach diesen Verschiedenheiten kann man sich über dorsal und ventral an der Kugeloberfläche orientieren, da die unpigmentirte Partie des Dotterpfropfes der Rückenfläche des Embryo zugekehrt ist."

Houssay ('90) observed the extension of the pigment to be "plus rapide sur le côté qui deviendra le dos de l'embryon."

While the point in question is not referred to, it is evident that the observations on the Frog, Triton, and Axolotl, as well as those of Kölliker ('79), on the Chick, and Lwoff ('92), on *Amphioxus*, accord perfectly with what I have found in *Amblystoma*. Morgan and Tsuda ('94) hold, however, that "in *Rana* the blastopore appears on the less pigmented and further developed side of the egg."

The stage shown (Pl. XX, Fig. 1) is soon followed by a sinking in of the cells on either side of the line, forming a depression which, in 8 hrs., becomes a crescentic groove (Fig. 2).

A corresponding stage of *Rana* is shown in Fig. 9. The blastopore (*bp.*) appears in the same region as in *Amblystoma*; very early the line of invagination (*bp.*) assumes the form of a more or less acute angle (Fig. 9), but to this angle but little significance can be attached beyond its slight bearing on the theory of concrescence.

As is well known, different opinions are held concerning the formation of the gastrula in *Amphibia*. A brief summary will define the views of a number of investigators.

The archenteron is formed by invagination, as held by Scott and Osborn ('79), Hertwig ('82), Schultze ('88), Perényi ('89), Jordan ('93), Morgan and Tsuda ('94).

These authors are, however, not agreed as to the precise method; the majority believe the process to be one of infolding (embolic invagination). Morgan and Tsuda consider it largely a process of overgrowth (epibolic invagination), while Jordan believes both processes are involved.

The archenteron is not formed by invagination, but by delamination, according to Moquin-Tandon ('76), Houssay ('90), Robinson and Assheton ('91), and Marshall ('93).

With the aid of the mechanical devices described, I have been able to follow certain cells, which from individual peculiarities could be easily distinguished, in their course from the point which they occupied at the rim of the blastopore, step by step, until they disappeared from view. It is evident, therefore, that there is either an infolding of the surfaces on either side of the line of invagination, or that the surface at the dorsal lip, is overgrowing the other, and at the same time infolding, giving such an appearance. Sections show that at this stage the ectoblast has overgrown the entoblast to a slight extent only, so that while there is some indication of epiboly, the appearance of sections and the actual infolding of cells observed on the surface can only be interpreted by considering the process as one of modified emboly.

The lateral extension of the groove soon reaches its limit, the ends turn toward the vegetative pole, and in 15 hrs. the crescentic blastopore has extended to the form shown in Pl. XX, Fig. 3. This is soon followed by the union of the ends completing the blastopore. At this time there is an infolding around the entire rim, the rate being most rapid at the dorsal margin, and gradually decreasing toward the ventral.

Fig. 7 represents a condition often observed where the blastodermic margin is defined at a much earlier period than in Fig. 3. A like condition was often found in *Rana* (Fig. 8) and in *Petromyzon* (Fig. 10).

The closure of the blastopore progresses gradually with the infolding. In 18 hrs. the condition shown in Fig. 4 is reached, when the external indications of gastrulation are beginning to fade. The blastopore has narrowed as a result of the unequal infolding. I am also inclined to believe that it is in part the result of mechanical pressure caused by the thickenings of the epiblast (*n.f.*) on either side of the dark hemisphere.

In a few hours the oval opening shown in Fig. 4 has been reduced to the slit-like aperture indicated in Fig. 5. This narrow slit is soon entirely closed at its center (Fig. 6), leaving an anterior opening lying just within the neural folds (*n.f.*), forming the neuropore (*np.*), and a posterior just without which persists and forms the anus (*a.*) as stated by Morgan ('80). This in *Amblystoma* is the usual method of closure, but it is by no means the only method. I have often observed a pear-shaped opening with the smaller end anterior, instead of the more usual dumb-bell outline. I have also followed the closure of the blastopore in *Rana palustris*, and am able to confirm the observations of Ziegler ('92), that the posterior portion of the blastopore persists as the permanent anus.

Hertwig ('92) states that in the Newt the "Urmundränder sich in einer von vorn nach hinten langsam fortschreitenden Richtung in der Medianebene zusammengelegt haben und verschmolzen sind."

Robinson and Assheton ('91) find in *Rana* that "the anus of *Rusconi* gradually diminishes in size by the conrescence of the ventral parts of the lateral lips."

Marshall ('93) confirms the observations of Robinson and Assheton, stating that "the reduction is effected, not by a contraction of the whole circumference of the blastopore, but by a folding together, or concrescence, of its lips in the median plane, beginning at the lower or ventral margin and proceeding upwards towards the dorsal margin."

Such a procedure I have never observed in *Amblystoma* or *Rana palustris*, although Jordan ('93) found it occurring in the Newt.

Before the neural folds are well defined (Fig. 5) the neural groove (*n.g.*) appears; in some cases it runs forward as a continuation of the slit-like blastopore, while in others it is not continuous, but an independent groove (Fig. 5). A second groove is often observed lying between the posterior end of the neural groove and the blastopore; often it arises at, and is continuous with, the dorsal lip of the blastopore.

2. THE ORIGIN OF THE MESOBLAST AND CHORDA.

The study of the early mesoderm may best be introduced by examining a meridional section (Pl. XXI, Fig. 1) of such an egg as that shown in Pl. XX, Fig. 7.

The segmentation cavity (*s.c.*) in most cases shows a radial symmetry, although occasionally its bilaterality is strongly marked, the roof being composed of from two to three layers of cells. The slight infolding shown in surface views is here observed to occur along a line where the entoblastic cells shade off into the smaller epiblast cells. I shall henceforth speak of this infolded blastodermic rim (*g.r.*) as the germ-ring, since I believe it to be homologous with a like thickening in the blastoderm of Teleosts and Elasmobranchs to which this term is generally applied. Just within and above the line of invagination there is a group of cells (*m.*) which are larger than the adjoining ectoblastic cells, and possess more pigment than the entoblastic cells. These cells, extending around the entire egg, form a ring which later gives rise to the mesoblast.

Fig. 2 represents a vertical section along the line *xy* of Pl. XX, Fig. 2. The roof of the segmentation cavity is somewhat thinner than in the preceding stage, being in some por-

tions but a single layer of cells. The dorsal lip (*d.l.*) of the blastopore is well defined, the ventral being indicated by a slight thickening (*g.r.*) at the opposite side of the egg. At the dorsal lip in the slight angle formed by the infolded epiblast there are a few loosely scattered mesoblastic cells (*m.*).

Passing now to Fig. 3, which represents a vertical section along the line *xy* of Pl. XX, Fig. 3, we observe a decided differentiation of the mesoblast (*mes.*) even before the infolding is well marked. These mesoblastic cells, in size, pigmentation, and reaction to stains, more closely resemble the cells of the epiblast than those of the entoblast.

In sections (Fig. 4) through a still later stage (between Figs. 3 and 4, Pl. XX), the cleavage cavity is much smaller, having been gradually reduced as the gastral cavity has extended. The roof of the gastral cavity is made up of two kinds of cells, which merge along a fairly well defined region. The cells nearer the blastopore partake of the character of the epiblastic cells, while toward the segmentation cavity they resemble those of the entoblast. The thin anterior wall of the mesenteron, as well as the communication often found between the two, lead one to infer that a part of the segmentation cavity may often become continuous with that of the mesenteron.

In the angle formed by the invaginated hypoblast the mesoblast (*mes.*) is better defined. Some distance below the dorsal lip of the blastopore is a second group, which is the ventral portion of the ring (ventral mesoderm).

There is, then, at even this relatively late stage, a continuous layer of mesoblast around the blastopore. Soon a division occurs at the dorsal lip, due possibly to the pressure of the chorda hypoblast against the epiblast. Whatever may be the cause, the fact is, that before the blastopore is entirely closed there is no longer mesoblast in this region, while it is present and well defined on either side of the chorda.

The later growth of the mesoblast is through the antero-lateral extension of the wings lying on either side of the chorda and the postero-lateral extension of the continuous zone at the ventral lip of the blastopore.

While the majority of observers have denied the existence of a continuous layer of mesoblast at the dorsal lip of the blastopore, there are certain observations scattered through the literature which show that its presence in some forms, at least, is an undisputed fact. Götte ('75), in *Bombinator*, finds all three layers existing at the dorsal lip when the first indications of gastrulation appear.

Schultze ('88) says concerning the existence of the mesoblast in this region in *Rana*, that "Schon auf dem Stadium der Beginnenden Gastrulation in der dorsalen Urdarmand drei Keimblätter existieren."

Houssay ('90) has found the same to be true in the *Axolotl*, and says: "L'étude de l'*Axolotl* confirme donc tout ce qui a été dit par Götte et Schultze, à propos des Anoures."

Perényi ('89) in *Bombinator* found the three layers of the epiblast to be folded in at the lip of the blastopore, the outermost becoming invaginated hypoblast, while the others gave rise to mesoblast.

Marshall ('93) states that in *Rana*, "At the lip of the blastopore, round its entire circumference, the three germinal layers, epiblast, mesoblast, and hypoblast, are indistinguishably fused together."

The chorda. — Concerning the origin of the *Chorda dorsalis* in *Amphibia*, no less than a score of investigators have recorded their opinions.

Among those who hold that the *chorda* is of entodermic origin are: Scott and Osborn ('79), Bambeke ('80), Hertwig ('82), Orr ('88), Houssay and Bataillon ('88), Robinson and Assheton ('91), and Jordan ('93).

Götte ('75) and Schultze ('88) dissent from the above, and maintain that it arises from the mesoderm.

When the infolding has progressed to the extent shown in Pl. XXI, Fig. 4, the outline of the *chorda* resembles the section of a cone. It consists of a single layer of invaginated hypoblast forming the roof of the gastral cavity (*g.c.*). As gastrulation proceeds, this layer of hypoblast extends and assumes an obovate form, consisting throughout of a single layer of columnar cells which on either side pass over into the

mesoblast. When the blastopore has narrowed to a slit, the chorda (*ch.*) consists of a single layer of hypoblast but few cells in width, as shown in Fig. 9.

Reconstructions show an irregular outline, the enlargements and constrictions often appearing in such order that they suggest the possibility of a metameric arrangement. The later development is essentially the same as observed in other Amphibia. The layer of columnar hypoblast folds up, and is gradually constricted until it lies free in the mid-dorsal region.

3. EXPERIMENTS.

Before considering the embryonic area, I wish to record the results of certain experiments.

The experiments were made on the eggs of *Amblystoma tigrinum* during March and April, 1893, in the Morphological Laboratory of the University of Michigan.

A method was sought by which I might be able to distinctly mark the surface of the egg and at the same time interfere as little as possible with normal development. After a number of methods were tried the following one was adopted: The outer envelopes are removed from the egg, which is then placed in a watch crystal on a bed of cotton, with barely enough water to cover it. The egg is then rotated until the desired point is uppermost, when it is punctured with an extremely fine hair held by forceps. A small quantity of the protoplasm oozes out and forms a minute extra-ovate, which remains attached to the egg. The eggs thus marked are transferred to watch crystals, in which they remain until the embryo is formed. A large mirror is fastened to the stage of a dissecting microscope; on this the watch crystals are arranged in series, and the eggs examined by means of an extension arm. The image of the opposite side may be thus observed during the entire period, from the time of marking until the embryo is formed. This is necessary, since the very small extra-ovates are easily detached.

Since to record these experiments in detail would require considerable space, I confine myself to a brief summary.

The center of the dark hemisphere was punctured in early and late segmentation stages, and the location of the extra-ovate observed when the embryo was formed. The results were variable. In 7 eggs the extra-ovate occupied a position near the median line, just beyond the region of the cephalic portion of the neural fold. In 5 cases it was located just within this fold; in 4, on the right side of the median line; and in 1, on the left.

The cause of these slight variations is probably due to the fact that punctures cannot be made at the same point in the different eggs.

From these experiments I conclude that the apical pole corresponds to the later head region of the embryo. This observation accords with what occurs in the Teleost egg, as stated by Professor Whitman in lectures on vertebrate embryology.

Schultze ('87) believes, however, that in *Rana* the apical pole represents the mid-dorsal region of the embryo.

Roux ('88^a), in the same form, holds that the apical pole forms the ventral side of the embryo.

Were it true that the apical pole later forms the ventral part of the embryo, we should expect to find considerable pigment in this region, as well as an area of smaller cells, which is not the case.

If at the first appearance of invagination a puncture is made in the dorsal lip of the blastopore, much variation occurs in the later position of the extra-ovate, probably owing to the difficulty in locating the median portion of the embryonic area. It will be recalled that one side of the groove may extend more rapidly than the other. If, however, the puncture is made at a time when the blastopore is crescentic or horse-shoe shaped, there is less difficulty in orienting the embryo. In all cases (17 eggs) the extra-ovate lies in the posterior third of the embryo.

Concerning the fate of the dorsal lip of the blastopore, we have but the observations of Schultze ('87) and Roux ('88^a). The former finds a defect in this region to later lie in the posterior portion of the embryo, while Roux finds that an injury produced here later lies near the transverse fold of the head.

If, when the blastopore has become horse-shoe shaped, punctures are made on the outside at either extremity, the extra-ovates generally (7 eggs) lie at the side of the slit-like blastopore, or are carried in (2 eggs). In 9 eggs markings at the ventral lip of the blastopore, when it had become circular, were found at the posterior end of the embryo.

The observations of Morgan and Tsuda ('94) show that the same is true in *Rana*; and further, if the puncture is made at the center of the vegetative pole, it later lies in the tail region of the embryo. Often it happens that the extra-ovate is infolded or overgrown.

In 13 cases, when the blastopore had become crescentic, punctures were made at the equator, on the opposite side of the egg. The extra-ovates later occupied a position on the ventral side of the egg, beyond the caudal end of the embryo. Roux ('88^a) states that a defect here is later found at the caudal end of the embryo.

4. THE EMBRYONIC AREA.

The older idea of von Baer was that the Frog's egg, like that of the other vertebrates, could be considered as made up of two parts, the germinal and nutritive, represented by the dark and light hemispheres respectively.

Pflüger ('83) holds that the embryo develops largely in the white hemisphere of the egg, but thinks it probable that the anterior portion of the medullary plate arises from the black hemisphere.

Schultze ('88), by following the path of certain local defects found on the surface of the egg, concludes that the embryo is formed entirely in the dark hemisphere.

Roux ('88) concludes from the fate of a number of artificial markings produced by a hot needle that the embryo develops almost exclusively from the inferior hemisphere. Hertwig ('92), from a study of abnormal forms, reaches practically the same conclusion.

Morgan and Tsuda ('94) state that they in the main point agree with Pflüger and Roux.

The results of my observations are : First, an area at the apical pole forms the basis of the head of the embryo.

Second, the area at the dorsal lip of the blastopore lies in the posterior half of the embryo.

Consequently, the anterior half of the embryo, at least, is formed from the material lying between the apical pole and the dorsal lip of the blastopore.

The formation of the posterior portion of the embryo is so intricate that I hesitate to attempt a description, realizing that more careful study is necessary before anything like a complete explanation can be offered. We know that two processes, that of overgrowth and infolding, are going on during gastrulation; that these vary in rate in the different portions of the blastoporic rim, the most active area being at the dorsal lip. This variable rate of infolding, in connection with the pressure necessarily resulting from the thickened areas of the medullary folds, make the closure of the blastopore an extremely complicated process.

That this closure may occur in a number of different ways is undoubtedly true, as the observations of Schanz ('87), Morgan ('89), Erlanger ('91), Robinson and Assheton ('91), Ziegler ('92), Jordan ('93), and others, amply testify. I cannot consider this or that variation as indicative of the method of embryo-formation, but rather as secondary modifications of the more primitive process of uniform convergence.

The above facts indicate that the greater portion of the body of the embryo is formed in the dark hemisphere by differentiation *in situ*, while the tail arises from the coalescence of the blastoporic lips.

5. THE PRIMITIVE STREAK AND GROOVE.

Throughout the literature scarcely two statements can be found which agree as to the nature and extent of the primitive streak. In the Amphibia this is especially true, as the following brief references indicate :

1. The primitive streak is in front of the blastopore, Alice Johnson ('84), Schultze ('88), Erlanger ('90).
2. The primitive streak lies behind the blastopore, Minot ('92).

3. The primitive streak represents the part of the blastopore between the neurenteric canal and anus, Schwarz ('89).

4. The primitive streak represents the entire fused area around the rim of the blastopore, Robinson and Assheton ('91).

The term "primitive streak" as is well known, was first applied to an opaque streak in the posterior portion of the area pellucida of the chick; this streak, according to Balfour, is due to the presence of a third layer, the mesoblast. The later authors have quite generally agreed that the primitive streak is the portion of the blastoporic area where all three layers are fused.

This area of fusion, as stated in a preceding page, extends around the entire blastoporic margin, so that in *Amblystoma* the basis of the primitive streak is a well defined ring. It is not, however, until the closure of the blastopore that the structure is generally referred to as such.

I wish here to refer to a structure which has been interpreted as a more or less exact criterion of the extent of the primitive streak, *viz.*, the "dorsal groove." It would indeed be a hopeless task to attempt the enumeration of synonymous terms which have been given by the various authors, or to record the many views regarding its position and extension.

In *Rana temporaria*, according to Hertwig ('82), the dorsal groove extends forward from the blastopore, while in the same form Robinson and Assheton ('91) find it extending backward from the blastopore. In the Newt Hertwig ('82) never finds the groove continuous with the edge of the blastopore, while Miss Johnson ('84) often finds this occurring. Jordan ('93) observed the primitive streak and neural grooves to later unite and form one structure. In a recent publication Bambeke ('93) has endeavored to show that the dorsal groove (*Rückenrinne*, *sillon médian*) represents the primitive groove and is the outward expression of the extent of the primitive streak. The author says:

"On peut admettre avec O. Hertwig, que le sillon médian ou dorsal (*Rückenrinne*) des Amphibies représente la ligne de suture, suivant laquelle, en vertu de leur rapprochement lent d'avant en arrière, les lèvres du blastopore se sont juxtaposées

et fusionnées. Le sillon médian est donc comparable à la formation désignée par Hatschek sous le nom de raphé gastrulaire (Gastrularaphé) des Ascidies, de l'Amphioxus et des Annélides."

These different views manifestly rest upon different conceptions of the extent of the blastopore and its method of closure.

A second source of confusion lies in the fact that the primitive, neural, and dorsal grooves are considered as one structure. In *Amblystoma* and *Rana palustris* there is a primitive groove, but this groove is not in front or behind the blastopore. It is the blastopore, or its remnant. In section, the line representing the area along which the lips have fused, is always present beneath it. A second groove is also often present, extending forward from the dorsal lip of the blastopore. It may appear as a slight depression just in front of the dorsal lip, extending in either direction, uniting the primitive and neural grooves, or it may be entirely absent. Sections through this groove show no line of fusion, nor in any way suggest that such has occurred.

The one fact which I wish to emphasize, is that this groove is in no way indicative of the extent of the primitive streak, but is a part of the neural groove, having arisen in precisely the same manner, while the primitive groove has an entirely different origin.

6. THE EPIBLAST AND MESOBLAST IN PETROMYZON.

A glance at the literature on this form shows a wide disparity of views concerning the process of mesoblast formation and extension. This, together with certain facts concerning the thinning of the roof of the segmentation cavity, preceding gastrulation, have led me to record a part of my observations.

In a late cleavage stage the roof of the cavity is composed of from two to three layers of cells, resembling the condition already noticed in *Amblystoma*; just before gastrulation the roof thins to a single layer of cells (Pl. XXI, Fig. 5), as stated by Schultze ('56), Scott ('81), and Shipley ('88). Kupffer ('90) and Hatta ('92), have likewise observed the thinning, but hold that the single layer is not reached.

A certain amount of this thinning may be due to a migration of the cells toward the equator, as held by some authors, yet I am satisfied that this is not the full explanation, since if the cells passed around the sides they should be recognizable from their smaller size. Moreover, it is impossible to interpret such appearances as shown in Fig. 5, except on one of the following hypotheses: Either these cells are wedging themselves in between the cells of the external layer, or they are being pressed out from between them. The former seems the more probable, since one must, at this time, think of an extension of the superficial layer rather than a reduction. Besides these cells possess coarser granules than the cells of the external layer, which accords perfectly with the former view, but not the latter.

Regarding the formation and extension of the mesoblast I have found no two authors in complete agreement.

Calberla ('78) states that the mesoderm arises through the division of the primary entoderm into mesoderm and secondary entoderm.

According to Scott ('81) the mesoblast is derived from two sources: One portion arises from the invaginated blastoderm, and a second portion is derived from the outermost layer of yolk cells. The latter completes the mesoblast on the ventral side of the body.

Nuel ('81) holds that a layer of hypoblast, lying between the layer forming the roof of the alimentary canal and the epiblast, gives rise to the mesoblast.

Shipley ('88) describes the mesoblast as arising from two bands of yolk cells which lie in the angles formed by the mesenteron and the epiblast. The differentiation begins in front and is continued backward.

Kupffer ('90) agrees with Shipley that the mesoderm extends from before backward, and when the region of the teloblast is reached its growth is supplemented by cells derived from this group.

Hatta ('92) believes the mesoderm to have a double origin. The part arising first is the "peristomial," which is found around the entire rim of the blastopore, save a point at the

dorsal lip. A second portion, forming the gastral mesoblast, arises on each side of the chorda.

Pl. XXI, Fig. 5, represents a vertical section through the area of deepest invagination in an egg, at the stage shown in Pl. XX, Fig. 10. Lying between the epiblast and the invaginated hypoblast is a group of indifferent cells (*m.*), which forms the basis of the mesoblast.

A later condition is represented in Fig. 6. The group of cells (*mes.*) designated (*m.*) in Fig. 5, are so highly differentiated that they plainly constitute a third layer. This layer extends around the entire periphery of the egg.

The majority of investigators have not recorded the existence of mesoblast at the dorsal lip of the blastopore, yet Scott ('81) has described it as follows: "In der dorsalen Blastoporuslippe vermehren sich die Mesodermzellen sehr rasch und bilden in der Mittellinie eine continuirliche Platte."

In Fig. 7, after Hatta, a later stage is shown. The segmentation cavity has disappeared, owing to the extension of the gastral cavity. At this time there is a group of cells at the ventral lip of the blastopore (*v.mes.*), which Hatta interprets as the ventral mesoderm, and further states that "mesoblast cells are budded out from all around the lip of the blastopore, except the dorsal median point, where the epiblast turns around to join the hypoblast."

I at once endorse this statement, but would add that in an earlier stage the mesoblast also exists at the dorsal lip; its division at this time is probably due to the pressure of the axial string of invaginated hypoblast. Thus, in *Petromyzon*, as well as in *Amblystoma*, there exists a well-defined layer of mesoblast around the entire blastopore.

In a still later stage (Fig. 8), there is a splitting off of certain cells from the layer forming the roof of the gastral cavity. These form a caudal knob (*tel.*) to which Kupffer ('90) has given the name "teleoblast." Kupffer expressly states that the cells take no part in the early formation of the mesoblast. "Ausgangspunkt der Bildung des Mesoderm ist also hier bei P. Plan. nicht der Teloblast, überhaupt nicht der Blastoporusrand." As will be observed from the figures, this stage is

much later than those in which the origin of the mesoderm has been described, and when there exists in the axial region the two sheets of so-called "gastral mesoderm."

7. GASTRULATION AND FATE OF THE GERM-RING IN AMIURUS AND LOPHIUS.

If the two eggs be compared just before gastrulation, we observe a decided difference in the relative amounts of blastodermic and yolk material. In *Amiurus* the mass of the blastodisc is to the mass of yolk as 1:8. In *Lophius* the relative proportions would be as 1:20. As the blastoderm extends there is a thinning of its central portion and a thickening and infolding of its margin. In a certain region the infolding is most pronounced, giving rise to a projecting tongue (Fig. 17) which first defines the long axis of the embryo. A point directly behind, at the edge of the thickening, will be henceforth referred to as the provisional hind-end of the embryo. We may also speak of the thickened blastodermic rim as the germ-ring.

Passing to later stages of *Lophius* (Fig. 18), we observe the first outlines of the embryo, terminating at its provisional hind-end in a caudal knob (*c.k.*). A dorsal groove, wanting in many Teleosts, is here present, extending from the anterior end of the embryo to a point just in front of the knob.

In *Amiurus*, Fig. 12, the head-end of the embryo is outlined; posteriorly, however, it is not yet defined. The dorsal groove is well marked and communicates with the blastopore. In this form the caudal mass is absent; there are, however, slight prominences on either side of the dorsal groove (Fig. 13) which may be its representatives. The discovery of this bifid condition of the posterior end of the embryo of *Amiurus* is due to Miss O'Grady, of Vassar College. It is of importance, as it is additional evidence of concrescence.

Later stages of *Lophius* (Figs. 19, 20) show the gradual extension of the germ-ring over the yolk and a corresponding differentiation and extension of the embryo; the dorsal groove has disappeared and the optic vesicles are outlined.

In *Amiurus*, Fig. 14, at a stage when relatively the same amount of yolk remains uncovered, we find the embryo less dif-

ferentiated, the dorsal groove is deep and wide, there is no indication of optic vesicles, and the embryo is obviously in a much earlier stage of development — a fact of no little theoretical interest as an illustration of the relation existing between embryo and yolk. If these forms be compared with *Coregonus* (Fig. 16), the effect is more pronounced, the blastopore being almost closed before the outlines of the embryo are distinct, recalling the condition observed in *Petromyzon* and *Amphibia*.

Fig. 21 represents a later stage of *Lophius*. The germ-ring shown in Fig. 20 constricts and is represented by the two portions surrounding the vesicles *c.v.* and *c.v.*' In some cases the anterior ring is united on either side with the provisional hind-end of the embryo. In others the tail apparently lies free, the ring joining the embryo at a point considerably anterior.

Fig. 13, Pl. XXI, represents a median sagittal section through an embryo at the stage just described. The provisional hind-end is well defined; the real hind-end being now at *g.r.*, and consisting of a mass of cells easily distinguished from the surrounding periblast (*per.*). The anterior ring is continuous with the provisional hind-end and the area enclosed by it is filled by a layer of periblast (*per.*), forming the roof of the cavity (*c.v.*). The walls of this cavity are lined with periblast. Lying immediately posterior to the vesicle (*c.v.*) is another (*c.v.*'), likewise lined with periblast. The layer of periblast forming the lateral walls and roof of these vesicles is of considerable thickness, while the portion forming the floor is of such extreme tenuity that it is often impossible to trace it throughout its entire extent. The roof of the anterior vesicle (*c.v.*) is formed by a layer of periblast only; over the posterior there is usually an additional layer of epiblast (*ep.*). I believe this drawing over, or extension, of the epiblast to be a secondary condition which offers no serious objection to considering the posterior ring as representing a portion of the original germ-ring. I have not been able to observe its formation in the living embryo, consequently am unable to say more.

Beneath the body of the embryo, at its posterior end, lies the peculiar vesicle (*K.v.*) to which Kupffer first called attention.

A later stage is represented, Pl. XX, Fig. 22, in which the anterior vesicle is much reduced in size; a slight constriction marks the boundary between the two portions of the germ-ring. Sections of this stage (Pl. XXI, Fig. 14) show the blastopore (*bp.*) to be also reduced in size. The periblast (*per.*) forming the yolk-plug is thickened; so far as I am able to determine the caudal vesicle (*c.v.*) does not open to the exterior.

The section Fig. 15 represents a stage when the closure of the blastopore is nearly complete; the continued thickening of the periblast is obvious; within it are a number of minute caudal vesicles (*c.v.*), which may represent the remnant of the anterior cavity (*c.v.*). The large cavity (*c.v.*') seems to have undergone no farther change during this interval. Its epithelial covering is on all sides continuous with the tail-end of the embryo. The size of Kupffer's vesicle has remained practically unchanged.

The embryo soon grows away from the constricted portion and the condition represented in Pl. XX, Fig. 23, results, when there is no longer communication as sections shown with the body of the embryo. Many variations exist in the distance between the posterior end of the embryo and the extra embryonic portion of the germ-ring depending upon the relative rate of growth of the embryo.

In an extremely late stage (Fig. 24), when the embryo has almost reached the larval condition, the ring is still present. Sections at this stage (Pl. XXI, Fig. 10) show that the size of the posterior vesicle is somewhat reduced. The periblastic ring, however, remains well defined. The section Fig. 11 is a more highly magnified picture of the region marked *per.* in Fig. 10, showing the aggregation of periblastic nuclei in this locality, also the continuous layer of epiblast (*ep.*). Since these are the latest stages in my possession, the fate of this part of the germ-ring remains undetermined.

The striking similarity of Kupffer's vesicle to the caudal vesicles *c.v.* and *c.v.*' and the close connection often observed, amounting in some cases (Fig. 14) to almost a complete fusion, lead one to infer that these vesicles have arisen from an originally single vesicle.

In the development of *Amiurus* a like phenomenon is observed, although less marked, owing to the existence of relatively less yolk. In the stage indicated in Pl. XX, Fig. 15, the embryo is well developed and in about the same stage as that of *Lophius* just described, having almost reached the larval condition. At the point where the tail of the embryo is attached to the yolk there is a streak (*g.r.*) extending ventrally to a point considerably removed as indicated in the figure. This is but faintly outlined in the unstained egg, but by surface staining it becomes strongly contrasted with the surrounding surface.

A section across this streak reveals the condition shown in Pl. XXI, Fig. 12. The periblastic layer is well defined and the nuclei are aggregated in groups in such a manner that it suggests a fusion of two portions. Lying above these two groups are two corresponding thickenings of embryonic tissue separated by a more or less distinct groove. In fact the entire appearance of the section at once leads one to suspect that it represents the fusion of two bands of embryonic tissue. I interpret it as due to precisely the same process which we have already noted in *Lophius* and which Miss Clapp has observed in *Batrachus*, namely: The embryo differentiates so rapidly that there is left behind a certain part of the germ-ring which never enters into its formation.

I believe this fact one of great importance in the conception of the formation of the embryo, and to which I shall revert presently.

8. THE MESOBLAST IN FISHES.

I have endeavored to determine the origin of the mesoderm in *Amiurus*, *Lophius*, and *Coregonus*, but as might be anticipated from the poorly differentiated condition of the cells of the different layers, these forms, like other Teleosts, are unfavorable for deciding critical points. While the majority of investigators first speak of the mesoblast when it is recognizable as two lateral bands, there are others who have considered it as primarily a continuous layer: Hoffmann ('81), Kowalewsky ('86), McIntosh and Prince ('90), p. 728.

Concerning the origin of the mesoblast in Ganoids the literature is meager. Salensky in describing its origin in Accipenser says:

"Pour étudier les premières phases de la formation du mésoderme, il faut remonter jusqu'à l'apparition du blastopore et de la cavité digestive primitive.

"Les cellules entodermiques adjacentes au bord du bourrelet marginal se divisent bien plus tôt que les cellules centrales et les cellules entodermiques avoisinant le pôle inférieur de l'œuf. Quand ces cellules entodermiques déjà réduites sont refoulées sous le bourrelet marginal, quelques-unes d'entre elles se maintiennent avec leurs caractères primitifs, sous les cellules entodermiques différenciées, pour former la paroi dorsale de la cavité digestive. Ce sont ces cellules entodermiques réduites, après s'être multipliées, qui constituent le premier rudiment du mésoderme."

Balfour and Parker give no account of its origin in Lepidosteus.

I have observed appearances in *Amia* which lead me to believe that the mesoderm forms as a continuous layer around the margin of the blastopore.

In Elasmobranchs there are a number of scattered observations to which I may briefly refer.

In the development of *Torpedo*, Swaen ('87) finds that "Dans toute l'étendue de la portion extra-embryonnaire du blastoderm, le mésoblaste a pour origine cette zone cellulaire périphérique dans laquelle les éléments épi- et hypoblastiques sont mélangés et qui établit la continuité entre l'épiblaste et l'hypoblaste primitif."

In the same form Schwarz ('89) found the mesoderm continuous around the entire blastoderm; to quote his words: "Hier ist, wie überhaupt im ganzen Randwulst, das Mesoderm seitlich mit dem Entoderm in kontinuierlichem Zusammenhang."

Kastchenko ('88), from a study of *Pristiurus*, *Scyllium*, *Torpedo*, and *Raja*, concludes that the mesoblast has a paired origin, but that later a continuous ring is formed. "Die freien Enden des peripheren Mesoblastes umwachsen nach und nach die ganze Keimscheibe und verwachsen mit einander am vor-

deren Rande der letzteren, indem sie einen geschlossenen Ring bilden."

The Zieglers ('92), in addition to the gastral mesoderm, describe a layer extending around the entire periphery of the blastoderm: "In den nächsten Stadien schreitet die Bildung des peripheren Mesoblastes rings um das ganze Blastoderm herum fort."

The above observations all point unmistakably toward a primitively unpaired condition of the mesoblast in Elasmobranchs. The formation of the axial mesoderm in some cases preceding the differentiation of the anterior portion of the peristomial is precisely what might be anticipated, since we would expect to find the process much retarded in this region, owing to the fact that through the great increase of yolk substance the most active area is far removed from that portion of the germ-ring which must be considered as representing the tail-end of the embryo.

I have purposely omitted referring to the origin of the mesoderm in *Amphioxus*, since Lwoff and E. B. Wilson have denied the existence of the pole cells, which, according to Hatschek, form the basis of the mesoderm.

9. GENERAL CONSIDERATIONS.

The evidence that the basis of the mesoblast in vertebrates is a closed peristomial ring may be briefly summarized as follows: I have demonstrated this fact in *Petromyzon* and *Amblystoma*. Its existence in Elasmobranchs is beyond question. Its presence in Teleosts has been repeatedly recorded. It explains certain peculiarities observed in Sauropsida and Mammals which have hitherto been unexplained, *viz.*, the formation of a part of the mesoderm at the periphery of the blastoderm, as observed in *Tryonix* and *Clemmys* by Mitsikuri ('91), and in *Sorex* by Hubrecht ('90).

I accordingly think the hypothesis well founded that the vertebrate mesoblast must be considered as primarily peristomial. The axial portion (axial mesoderm) in Amphibia has been carried in by invagination; while in forms containing a great amount of yolk substance, and where overgrowth has replaced invagination, the axial position is due to conrescence.

Concrescence.—Following the observation of Kowalewsky ('71), who considered the dorsal groove in the Frog, *Amphioxus*, and Ascidians as representing a continuation of the gastrula invagination, the papers of His ('76) on the Teleost and Rauber on the Chick clearly formulated the Theory of Concrescence, the evidence of this process in Amphibia being based upon the statement of Kowalewsky.

The presence of this groove has been considered by a number of later writers as evidence of concrescence.

Since I have already dwelt upon this point (p. 376), I refer the reader to the criticism there offered.

According to Roux the Amphibian embryo is formed by concrescence in the following manner: "Das Material zur Bildung der Medullarplatte jederseits durch seitliches Herabwachsen vom Äquatorrande aus auf die Unterseite des Eies geschoben wird, und dass diese von beiden Seiten her entgegenwachsenden Platten unten in der Medianlinie mit einander verschmelzen. Diese Verschmelzung findet successive und zwar in cephalocaudale Richtung statt."

This conception is based largely upon the experimental determination of the fate of the cells at the dorsal lip of the blastopore, which region, according to Roux, represents the head of the embryo.¹ If this one observation becomes an established fact, it will afford very conclusive evidence of concrescence. It must at present, however, be considered as a tentative hypothesis, since the observations of Schultze ('88), as well as my own experiments are far from confirmatory.

From a study of pathological forms Hertwig ('92) likewise holds that the embryo is formed by concrescence. "In der Rückenlinie erblicke ich die Nahtlinie, in welcher bald nach dem Beginne der Gastrulation die Urmundränder sich in einer von vorn nach hinten langsam fortschreitenden Richtung in der Medianebene zusammengelegt haben und verschmolzen sind." I am unable to discover whether Hertwig maintains two altogether different processes in Triton and the Frog, or whether he retracts the belief which he previously stated ('82),

¹ The late experiments by Morgan (*Anat. Anz.* 1894, p. 698) confirm the observations of Roux.

viz., "Nach vorn vom Urmundspalt und in geringer Entfernung von ihm senkt sich die Oberfläche des Eies zu einer kleinen Furche ein, die mit der Längsaxe des Eies zusammenfällt. Sie soll als Rückenrinne bezeichnet werden. Mit dem gleichgerichteten Urmundspalt fließt sie weder Anfangs noch auch später zusammen, sondern bleibt von ihm durch einen queren Wall getrennt, wodurch deutlich bewiesen ist, dass beide Bildungen in ihrer Genese vollkommen unabhängig von einander sind." Farther, p. 307: "Wenn die Primitivrinne der Vögel, wie jetzt vielfach angenommen wird (Gasser, Rauber, Braun) als Verschlussstelle des Urmundes angesehen werden muss, so entspricht sie dem Blastoporus der Amphibia, welcher später ebenfalls zu einem kurzen Langsspalt auswächst, dann aber kann sie nicht mit der Rückenrinne der Tritonen verglichen werden. Denn die letztere bildet sich vor dem Blastoporus, in einer Gegend, wo derselbe niemals gelegen hat, und ist von Anfang an durch einen Wulst von ihm getrennt. Das ist der Grund, warum ich den Namen Primitivrinne nicht für sie gewählt habe."

If the figures of abnormal embryos given by Roux ('88^a), Hertwig ('92), Morgan and Tsuda ('94), are compared, we observe marked differences in the extent of the blastopore. According to those given by Roux, its anterior limit is at a point just within the transverse medullary fold. Hertwig's Figs. 8 and 15, Pl. XVI, would indicate that it must extend far beyond, even to the region occupied by the sucking discs. In Figs. 17, 18, and 19 by Morgan and Tsuda the blastopore cannot be interpreted as extending even to the posterior border of the transverse fold.

These differences, together with the very great individual variations, make the evidence from this source very unsatisfactory. For my own part, I question the entire evidence adduced from pathological forms.

Minot's ('92) principal reason for believing concrescence to occur in Amphibia is the assumption that the dorsal lip of the blastopore, or rim of the blastoderm, becomes farther and farther removed from the segmentation cavity. Were this the case, it would be no more evidence of concrescence than of

overgrowth. A comparison of successive stages during gastrulation shows a continual diminution of the segmentation cavity, due to the extension of the gastral cavity, the part farthest removed from the dorsal lip being the last to disappear.

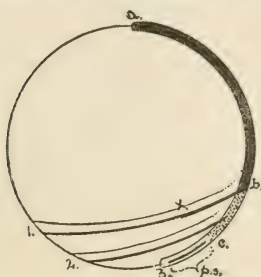


FIG. 1.

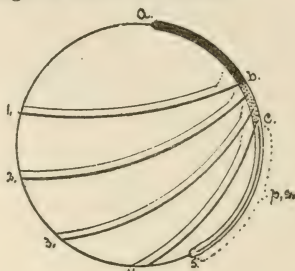


FIG. 2.

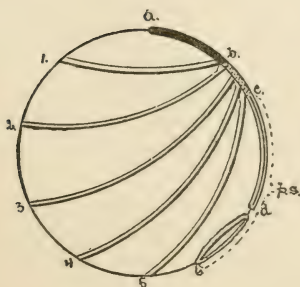


FIG. 3.

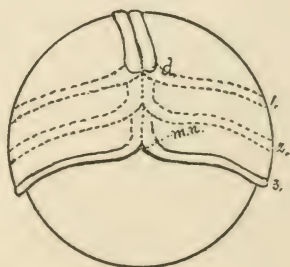


FIG. 4.

FIG. 1. — Diagram showing the formation of the embryo as observed in Amphibia.

FIG. 2. — Ditto in *Coregonus* and most Teleosts.

FIG. 3. — Ditto in certain Teleosts (*Batrachus*, *Amiurus*, *Lophius*) and Elasmobranchs.

FIG. 4. — Ditto in Chick after Rauber (modified).

The portion of the embryo (*a.b.*) formed by differentiation *in situ* is represented in black; that formed through backward extension, or overgrowth (*b.c.*) by dots, while the extent of concrescence forming the primitive streak is represented by the heavy line *p.s.*

The successive positions occupied by the germ-ring, or thickened blastodermic rim, are represented by 1, 2, 3, 4, etc.

These observations are the principal ones thus far brought forth to support the theory of concrescence in Amphibia, and to my mind they are far from convincing.

The accompanying diagrams are introduced to illustrate the modification arising in the formation of the embryo through a

constant increase in yolk, as compared with the mass of embryonic material.

In Fig. 1 I have attempted to represent the method of embryo formation in Amphibia, based on the study of *Amblystoma*. At a time when the blastopore extends around the entire blastodermic margin (1 *b*.) the first outlines of the embryo are barely visible; this portion of the embryo, extending to a point somewhere in the vicinity of *b*, is the part which is to be regarded as having formed through differentiation *in situ*.

Successive stages in the closure of the blastopore are represented at 1, 2, and 3. The closure in the earlier stages is effected through a convergence of the entire margin, which gradually changes to a lateral convergence, the margins finally coalescing to form the primitive streak (*p.s.*). From the position of the primitive streak one naturally infers that the ventral portion of the blastoderm must pass over the yolk most rapidly; the explanation probably lies in the fact that the effect of overgrowth is here least obscured by infolding. In order to comprehend the backward extension of the embryo from *b* to *c*, it is necessary to recall that at the dorsal lip of the blastopore there is an area in which the cell activity is greatest; otherwise the decided infolding at this point would appear incompatible with such an extension.

If we next consider a condition Fig. 2 (*Coregonus* and other Teleosts), we find the embryo has reached the same stage of differentiation as in Amphibia at a time when the blastoderm has extended over about one-third of the yolk. In other words, the embryo differentiates at a relatively earlier period than in Amphibia.

With the change in quantity and quality of yolk a corresponding modification has arisen in the process of gastrulation; there is no longer as extensive an infolding as in the preceding forms, while overgrowth must be considered as playing a more important part. We must also bear in mind the fact that the most active or formative area at *b* is farther removed from that portion of the germ-ring destined to form the tail of the embryo; so that during the time the germ-ring is passing over the yolk (1, 2, 3, 4) the embryo becomes well differentiated.

It is thus perfectly obvious that if this portion of the germ-ring ever enters into the formation of the embryo it must do so at a relatively later period than in Amphibia. The process by which the greater portion of the germ-ring is converted into embryo is an antero-posterior coalescence (conrescence) of its lateral portions, forming the primitive streak (*p.s.*). In accepting conrescence as the method, I adopt the view of His, that the head is practically a fixed point. In this diagram I have also represented the head of the embryo as arising at the apical pole. This is based directly upon the unpublished observations of Professor Whitman. The extent of the backward growth of the embryo through proliferation is difficult to determine. I have represented it as extending from *b* to *c*, rather to illustrate the fact that a certain portion is thus formed than to indicate its exact proportional extent.

Fig. 3 represents a still greater increment in the mass of yolk, as compared with the blastodisc (*Amiurus*, *Lophius*, *Batrachus*, and *Elasmobranchs*). The first outlines of the portion of the embryo (*ab*), which is to be considered largely the result of a differentiation *in situ*, appears at a time when the blastodisc covers a smaller portion of the yolk than in the forms represented in Fig. 2, its margin being indicated by the line *1b*. The successive positions occupied by the germ-ring and corresponding elongation of the embryo are indicated, as in the preceding, by the numerals 1, 2, 3, 4. When the region indicated by 5 is reached the ring constricts, leaving a part of the embryo (*6b*) behind. This is due to the fact that the formative area is so far removed from this portion that it cannot pass over the yolk before the embryo is formed.

This peculiarity, first observed in *Batrachus* by Miss Clapp ('91), and which I have observed in *Amiurus* and *Lophius*, illustrates the manner in which the "yolk blastopore" (Balfour) of *Elasmobranchs* has been formed, and offers a complete explanation of its morphological significance.

In a rare form of the Chick blastoderm, described by Whitman ('83), and which I have illustrated in Fig. 4, there was an extension of the primitive streak (*p.s.*) beyond the end of the tail (*d.*) to the marginal notch (*m.n.*). This condition was re-

garded by the author as evidence of concrescence. Considered in the light of the above facts, this view receives confirmation.

Owing to the enormously increased yolk, the germ-ring is cut off much earlier than in the forms represented in Fig. 3, having passed scarcely beyond the region of the equator.

With the increment of yolk there is a proportional increase in the extent of the primitive streak, until a certain point is reached, when it suffers reduction through the cutting off of the posterior portion of the embryo; so that in the Chick the anterior portion only of the primitive streak is present, the posterior portion being represented by the line extending from the tail-end of the embryo (*d.*) to the marginal notch (*m.n.*), plus the part represented by the rim of the blastopore. In the discussion of homologies it is all-important that this conception be kept in mind.

From a comparison of the above forms we observe that in Amphibia the greater portion of the embryo is formed through differentiation *in situ* and overgrowth, concrescence being confined to a limited region.

With the increase in yolk (most Teleosts) a greater extent (*p.s.*) of the embryo is formed by concrescence, the part arising through differentiation *in situ* and overgrowth undergoing a reduction. Through a still greater increment of yolk (certain Teleosts, Elasmobranchs, Aves) there is a corresponding increase in the extent formed by concrescence, while differentiation and overgrowth are of minor importance.

We conclude that the primitive method of embryo formation in vertebrates is through differentiation *in situ* and overgrowth, concrescence being a secondary method of uniting the lips of an elongated blastopore, this elongation having arisen through an increase of yolk.

The general remarks on the position and growth of the embryo, especially the Telostean and Avian, are largely the direct outcome of views expressed by Professor Whitman in his unpublished lectures on vertebrate embryology, and I gladly acknowledge my indebtedness to him.

10. THE NEURAL FOLDS IN AMBLYSTOMA.

The earliest stage in which the foundations of the neural folds are visible is represented in Pl. XX, Fig. 4. A transverse section shows slight lateral thickenings of the epiblast which, in a few hours, give rise to the bands (*n.f.*) shown in Fig. 5; these bands arise independently, and, so far as I am able to determine, differentiate *in situ*. They are later united anteriorly by a transverse thickening which forms the cephalic portion of the continuous fold. Clarke ('80) describes the folds as extending forward until they meet at the opposite end of the egg. Transverse markings such as shown in Pl. XXII, Fig. 9 (Necturus), are occasionally present in the neural plate at this time; to these, however, I give little emphasis, since I believe in most cases they are foldings between the myomeres, which at this time are beginning to form.

Pl. XX, Fig. 6, and Pl. XXII, Fig. 1, represent respectively the posterior and anterior portions of the same egg a few hours later. The neural bands have united to form a continuous fold, the cephalic portion having become especially prominent; the neural groove has appeared and extends to a point just within the cephalic fold. At the anterior end of the neural plate, on either side of the neural groove, is a slight depression, which in general outline is more or less circular, and in many cases pigmented; although by no means constant, their occasional presence is suggestive, since, as we shall see later in other forms, these areas form the bases of the paired eyes. A section of such an embryo is shown in Pl. XXII, Fig. 17: the foundation of the nervous system is a broad, thickened plate of epiblast, consisting of the epithelial (*ep.*) and sensory layers (*s.ep.*); beyond the region of the folds (*n.f.*), the sensory epiblast thins to a single layer of cells. Beneath the median portion of the neural plate there is a single layer of hypoblast (*hy.*) which forms the basis of the chorda (*ch.*). On either side of the chorda the sheets of so-called gastral mesoblast (*mes.*) are well defined, although not yet split into somatic and splanchnic layers. Just within the neural folds, on either side of the median line, are the depressions (*o.p.*) described above,

made up of narrow elongated cells which are deeply pigmented in their outer ends.

With the elongation of the embryo the neural folds become more prominent (Pl. XXII, Fig. 2). They are widely separated anteriorly, while posteriorly they are beginning to close. Certain markings which might be interpreted as neuromeres are often observed in the neural folds, yet their arrangement is decidedly irregular, and one is led to believe that they indicate nothing more than artifacts caused by the killing reagents.

The folds close more rapidly at the anterior end, so that in the succeeding stage (Fig. 3) they are about the same distance apart, throughout their entire length; in the cephalic region of the embryo, on either side there appear slight swellings. The anterior of these is the forerunner of the primary fore-brain, while the second represents the common foundation of the second and third cerebral vesicles. The folds soon close cephalad along their entire length. Occasionally I have seen the folds meet first at a middle point, as described by Clarke ('80).

In Fig. 4 the folds have closed, the outline of the embryo is well defined, the cerebral vesicles are distinct, the myomeres are differentiated throughout the greater portion of the embryo. The cranial nerves are forming; in short, the embryo is taking on those peculiarities which enable us to speak of it as a larva. I wish now to return to the consideration of certain features but faintly foreshadowed in the development of *Amblystoma*, namely:

II. THE OPTIC VESICLES.

Many attempts to explain the inverted position of the retina have led to the hypothesis that the vertebrate eye must have been located, originally, within the brain, Lankester ('80), Balfour ('85); or, as Beard ('88) stated: "Most of us now accept the view of Balfour, Carrière, and others, that the eyes were once structures opening dorsally on the surface of the unclosed neural plate."

While this was undoubtedly quite generally accepted, there were no observations to this effect until I showed in a prelimi-

nary paper ('93), the substance of which is here repeated, that this was precisely the case in *Rana palustris*. Soon afterward Locy ('93) found the same in the Shark.

It should not be said, however, that we were wholly without observations pointing toward an earlier differentiation. Bischoff, Kölliker, His, Van Beneden, and others have figured the early appearance of the optic vesicles in mammals. Heape ('84) finds in an early stage of the Mole, where the neural folds are closed along the center of the embryo, "at the anterior end the floor of the neural groove, on either side, is swollen, and on the outer and anterior edge of the two masses a deep narrow groove indicates the commencement of the formation of the optic organs."

Keibel ('89) describes a like condition in the embryo of the Guinea-pig. This apparently precocious development of the eyes in mammalia, showing no differentiation beyond the fact that depressions are present, is probably due to the retarded closure of the cephalic portion of the neural groove, and can scarcely be considered as a fact of phylogenetic significance.

Whitman ('89) discovered that in *Necturus* there is a very early appearance of the eye, "its basis being discernible as a circular area — after treatment with osmic acid followed by Merkel's fluid — long before the closure of the neural folds of the brain."

Through the kindness of Professor Whitman I have been able to study the development of the eyes in this form. In the stage (Fig. 10) corresponding closely to that described, the embryo is well defined, the closure of the neural folds having taken place posteriorly, and closely approximated well up toward the region which later forms the head. At the anterior end of the embryo, on either side, slight evaginations are visible. A section through these vesicles (Fig. 15), shows more numerous mitoses in these regions, as well as a marked migration of the nuclei toward the periphery. This is the earliest indication I have been able to make out satisfactorily. In Fig. 11 a later stage is represented, in which the optic vesicles are much better defined. Sections (Fig. 16) at this time show a continued migration of the nuclei, together with a thinning

of the wall until the optic vesicle is reduced to a single layer; meantime the external layer of the epiblast in this region has divided. In a few cases paired depressions have been found in earlier stages (Fig. 9). Sections, however, reveal nothing in the way of histological differentiation, and one might justly consider them as artifacts. The only thing which would dispel all doubt as to their meaning, would be to find some form in which they are so well marked that they may be traced, step by step, through the phases of involution of the neural plate, to the future optic vesicles. This condition is perfectly fulfilled by *Rana palustris*. In an early embryonic stage (Fig. 5), when the neural ridges are just forming, and are widely separated, paired depressions appear just within the cephalic folds, on either side of the median line. These areas are sharply contrasted with the surrounding parts by a much deeper pigmentation. A transverse section through them is shown in Fig. 12. The hypoblast (*hy.*) is a single layer forming the roof of the mesenteron. In the median line there is a fold which is the end of the chorda. In many cases this extends beyond the region of the neural fold, and is a point of theoretical interest. The mesoblast (*mes.*) consists of a single layer of cells, which passes insensibly into the axial region, where all three layers are fused. The sensory layer of the epiblast (*s.ep.*) exists as two lateral thickenings, united by the thinner median portion.

In *Amblystoma* and *Necturus* the superficial layer of the epiblast cannot be distinguished beyond the region of the neural folds, while in *Rana* it extends over the entire plate, as shown in the figure. In this layer the optic pits are formed. In addition to the fact that these areas are sharply defined by the presence of pigment to which more or less importance may be attached, they are further remarkable in that the elongation of the cells and the position of nuclei are indicative of a considerable degree of histological differentiation. Between these areas the cells are undifferentiated and resemble those of the superficial epiblast in other parts of the embryo. Sections further posterior show the cells of this layer to be uniform. Fig. 13 represents a section through a later stage, when the folds are still

widely separated. There is but little change in the histological character of the cells, except that their boundaries are less distinct. Owing to division and loss of yolk, the cells of the embryo have gradually become smaller and more compact. The originally broad space enclosed between the neural folds has undergone a constant reduction in size; a rapid closing of the anterior portion has taken place, so that along the entire length of the embryo the folds are approximated.

A section at this stage is represented in Fig. 14. The canal is elliptical owing to the slight evagination of the optic areas which are the forerunners of the optic vesicles. Their bilaterality is indicated by the distribution of pigment only, and one might justly consider them as derived from a common basis. A marked migration of the pigment has taken place; instead of being located at the ends of the cells as in earlier stages, it is found between them and nearer the periphery. The nuclei have likewise undergone a further migration toward the surface, so that the cells of the superficial layer have completely lost their identity.

It is of interest to note that in *Petromyzon* at a stage corresponding closely to the above, Kupffer ('90) has observed that an unpaired basis for the eyes is present as the following quotations show: "In der Mittelebene zeigt sich gar kein Merkzeichen welches auf Duplicität der Anlage deutet, dieselbe ist vielmehr zunächst eine unpaarige. . . . Die später paarige Erscheinung des Organs wird nur darin angedeutet, dass in den lateralen Regionen der Erweiterung beiderseits Mitosen auftreten, die am Boden fehlen."

During the later stages of development the walls of the vesicles become thinner so that just before they invaginate to form the optic cups they consist of but a single layer of elongated cells with their nuclei located in their peripheral ends. A continued dispersion and migration of the pigment takes place, and at this time is more abundant in the stalk than in the portion which will later form the optic cup. The later development of the vesicles in this form is essentially the same as in *Necturus* and *Amblystoma*. Since these stages have been carefully studied by Professor Mall ('93) a further description would be superfluous.

12. THE OLFACTORY AND ORAL GROOVES IN RANA.

In Pl. XXII, Fig. 6, an embryo of 70 hrs. is represented. The neural folds are anteriorly wide apart. At this stage paired grooves (*ol.g.*) arise in the cephalic portion of the neural fold; these grooves are short, shallow and deeply pigmented.

A later stage is represented in Fig. 7 in which the neural folds are united along their entire length. The distal ends of the grooves have extended antero-laterally, while the proximal ends have been united by the closure of the neural folds causing the V-shaped arrangement shown in the drawing.

In Fig. 8, the grooves have reached their maximum extension, having grown antero-laterally until their distal ends reach well over toward the anterior border of the primary fore-brain. At this time the pits form at the distal ends of the grooves and the nose becomes localized. The lines of pigment are exactly similar to, and continuous with, the line which indicates the suture formed by the closure of the neural folds.

The mouth usually arises as an independent median invagination; in some cases, however, it shows extremely interesting peculiarities.

In the development of the nervous system of *Rana* the closure of the neural folds occurs in two ways; the most frequent is by a median approximation of the lateral portions of the neural folds, together with an antero-posterior folding of the cephalic portion. Often the antero-posterior folding does not occur, or if so, to a slight extent only. When this occurs a very shallow pigmented groove is formed just at the anterior end of the neural groove.

At a time shortly after the folds have begun to approach posteriorly the appearance of the oval groove is foretold by a very slight depression at the anterior end of the neural groove. This would appear in a somewhat earlier stage than Fig. 6. With the progressive closure the groove extends farther anteriorly (Figs. 7 and 8); in the latter it reaches just beyond the region of the primary fore-brain. The oval invagination is now outlined and localized in the distal end of the groove in a manner quite similar to the olfactory pits.

Concerning the significance of these structures it can only be said that from our knowledge of the organ in other groups we should scarcely be warranted in considering them of phylogenetic importance; yet it cannot be said that these peculiarities are without hypothetical interest.

13. PARAPHYSIS AND EPIPHYSIS IN AMBLYSTOMA.

From the numerous late researches it seems probable that in most forms there are at least two median outgrowths in the roof of the encephalon.

The posterior of these, the epiphysis, arises according to the majority of observers, among whom I may name Béraneck ('87), Strahl and Martin ('88), Françotte ('88), Selenka ('90), and Klinckowstrom ('93), as a median unpaired evagination in the roof of the thalamencephalon; this structure soon gives rise to a secondary, the parietal vesicle or pineal eye.

Others hold that instead of considering one of these vesicles as secondary they are to be considered as the representatives of two homodynamous structures, Leydig ('90), Hill ('91), Locy ('93).

Again there is found a group of several vesicles arising in this region, Duval and Kalt, Prenant, and others.

The anterior, or paraphysis, generally arises slightly anterior to the line which marks the boundary between prosencephalon and thalamencephalon. Concerning the fate and function of this organ there is much difference of opinion. Françotte ('88) derives from it a portion of the future choroid plexus. Selenka ('90) suggests that it may be the rudiment of an ancestral ear, while Leydig ('90) finds, instead of a single vesicle, a group of five which later come into such close relation to the epiphysis that Spencer ('84) figured both as one structure.

In a brief preliminary ('92) I showed the epiphysis and paraphysis to be entirely independent structures in *Amblystoma*. In the following pages I repeat the principal facts:

The first trace of the epiphysis is in a 5 mm. embryo where it shows, in section, as a crescentic evagination in the roof of the thalamencephalon. The lateral walls are formed of several layers of cells, while the dorsal, which comes directly in con-

tact with the superficial layer of the epiblast is but a single layer. The nuclei undergo a marked migration toward the periphery, and in this respect the appearance is strikingly similar to the condition found in the optic vesicles, which at this time are strongly evaginated. The presence of pigment at the inner ends of the cells is also a significant fact.

From this time until the formation of the lens in the lateral eye, the epiphysis increases in size, its cavity becomes elliptical and is in wide communication with the thalamocoele.

At the time of the invagination of the lens there appears in the posterior portion of the roof of the prosencephalon a second median outgrowth which is probably homologous with the paraphysis described in Reptilia by Selenka.

In a 12 mm. larva the paraphysis is much elongated; lateral diverticula appear at its distal end while the cavity is obliterated proximally in a manner analogous to that which occurs in the epiphysis. The changes are more pronounced in 14 mm. larva where it has assumed a digitate appearance and bears a striking resemblance to the true choroid plexus of the lateral ventricles. Hill ('94) has described the paraphysis in *Amia* and believes that it likewise later forms a part of the choroid plexus.

The two structures in Urodela never come into close relation, as in Reptilia, but remain widely separated. That the paraphysis is indeed a peculiar organ, very similar in development to the epiphysis, is shown not only by Selenka but also by Leydig in his final memoirs, where from its exceptional mode of development, the name "anterior epiphysis" is suggested. The hypothesis of Selenka that it is a degenerate sense organ, is certainly questionable; much more that its ancestral function was audition.

The variation in the point of origin of the paraphysis, and its formation at a much later period than the epiphysis, would seem to indicate a less important ancestral function. The phylogenetic importance of the epiphysis is certainly indicated by the fact that it is formed at a fixed point throughout the vertebrate phylum. While it must be admitted that we are without evidence sufficient to warrant us in making any

very definite statement, since if the organ is rudimentary its development may be retarded, yet its ontogeny indicates that it arose at a time when a neural canal was first formed. If we admit the hypothesis, which I believe I have proved, namely, that the lateral eyes are present as a pair of depressions in the cephalic neural plate, we might anticipate that at the phylogenetic period when they become implicated by the closure of the neural folds a median eye would arise and become most highly functional during the period when the lateral eyes were non- or least functional. This might explain the origin of the epiphysis as an unpaired organ; the circumstances which give rise to another sense organ, if such the paraphysis be considered, are extremely difficult to explain. In my preliminary note I suggested the above hypothesis as one of the possible explanations of the origin of a median unpaired visual organ.

Concerning this hypothesis Professor Béraneck ('92) says:

"Quel a été le rôle de l'œil pariétal? Pourquoi s'est-il développé? Eycleshymer cherche à résoudre ce problème en disant que, lors de la fermeture du tube médullaire, les yeux pairs — auparavant directement influencés par la lumière — perdirent d'une manière plus ou moins complète leur fonction. Cette perte d'activité fonctionnelle fut compensée par l'apparition d'un œil dorsal impair, qui commença à s'atrophier, lorsque les yeux pairs eurent reconquis leur prépondérance.

"Cette explication ne me satisfait pas, je l'avoue. Tout d'abord, j'ai peine à comprendre que les yeux pairs perdent leurs fonctions pour les reprendre plus tard, alors qu'un autre organe visuel est apparu entretemps pour les suppléer. Puis ce rôle de suppléant, joué par l'œil pariétal, s'accorde peu avec la persistance de cet organe dans un groupe de Vertébrés aussi élevé que celui des Sauriens, groupe dont les yeux pairs sont fort bien organisés."

Although laying but little stress upon this view, as expressly stated in my preliminary, I still maintain that there is nothing unphysiological in postulating the decrease of function in a given organ for a certain length of time and its gradual revival under favorable environment. Lankester ('80) and Balfour

(85) have both expressed such views concerning the development of the paired eyes.

The fact that Béraneck, Strahl and Martin, Françotte, Prenant, and others have found a transitory nerve passing to the pineal organ, might even be considered additional evidence of its having been a supplementary organ. Why it should have continued functional in some forms as indicated by a rich nerve supply, and in others degenerated, is an entirely secondary question.

The origin of the pineal organ, in the manner described by Leydig and Hill may indicate that it is derived from paired sensory areas. The accessory vesicles described by Locy are also very suggestive, and later investigation may prove that the parietal eye arises from the coalescence of two such structures. The recent work of Kupffer, however, on the origin of the secondary vesicle in *Petromyzon*, in which form the structure is functional, if we may judge from the rich nerve supply, shows nothing which can be interpreted as indicating a paired origin.

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|-------------|---------------------------|--------------|----------------------|
| <i>a.</i> | Anus. | <i>n.f.</i> | Neural folds. |
| <i>bp.</i> | Blastopore. | <i>n.g.</i> | Neural groove. |
| <i>ch.</i> | Chorda. | <i>n.p.</i> | Neuropore. |
| <i>ck.</i> | Caudal knob. | <i>o.g.</i> | Oral groove. |
| <i>cv.</i> | Caudal vesicle. | <i>ol.g.</i> | Olfactory groove. |
| <i>cv'.</i> | Secondary caudal vesicle. | <i>o.p.</i> | Optic pits. |
| <i>dl.</i> | Dorsal lip of blastopore. | <i>per.</i> | Periblast. |
| <i>ep.</i> | Epiblast. | <i>s.c.</i> | Segmentation cavity. |
| <i>g.c.</i> | Gastral cavity. | <i>s.ep.</i> | Sensory epiblast. |
| <i>g.r.</i> | Germ-ring. | <i>tel.</i> | Teloblast. |
| <i>hy.</i> | Hypoblast. | <i>v.p.</i> | Vegetative pole. |
| <i>K.v.</i> | Kupffer's vesicle. | <i>yk.</i> | Yolk. |
| <i>m.</i> | Mesoblast anlage. | <i>y.p.</i> | Yolk plug. |
| <i>mes.</i> | Mesoblast. | | |

EXPLANATION OF PLATE XVIII.

All figures from Amblystoma punctatum.

FIG. 1. Egg with enclosing membranes just after laying ($\times 8$).

FIG. 2. Superior hemisphere of egg 2 hrs. after laying, showing "light spot," within which lies the first polar body ($\times 15$).

FIG. 3. Superior hemisphere 4 hrs. after laying, showing eccentric aggregation of pigment, also presence of second polar body ($\times 15$).

FIGS. 4-12. Successive stages of cleavage from living egg ($\times 15$).

FIGS. 13-18. Successive stages of cleavage in superior hemisphere of living egg, showing order and time of appearance ($\times 15$). Heavy black line indicates the first cleavage; heavy black broken line, second cleavage; blue line, third cleavage; heavy red line, fourth cleavage; black dotted line, fifth cleavage; red dotted line, sixth cleavage; light black line, seventh cleavage. Numerals indicate time of first appearance of the furrows.

FIG. 19. Meridional section of egg in stage corresponding to Fig. 7, showing early segmentation cavity ($\times 20$).

FIG. 20. Meridional section through the centers of least and most densely pigmented areas in stage corresponding to Fig. 9 ($\times 30$).

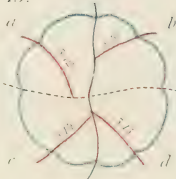
FIGS. 21, 22. Meridional sections through same areas, showing thickening of roof in densely pigmented area, and thinning at opposite side ($\times 35, 40$).

1



b
d

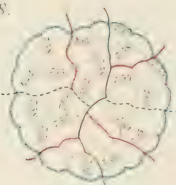
15.



2.



18.



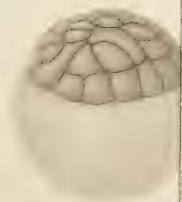
22.

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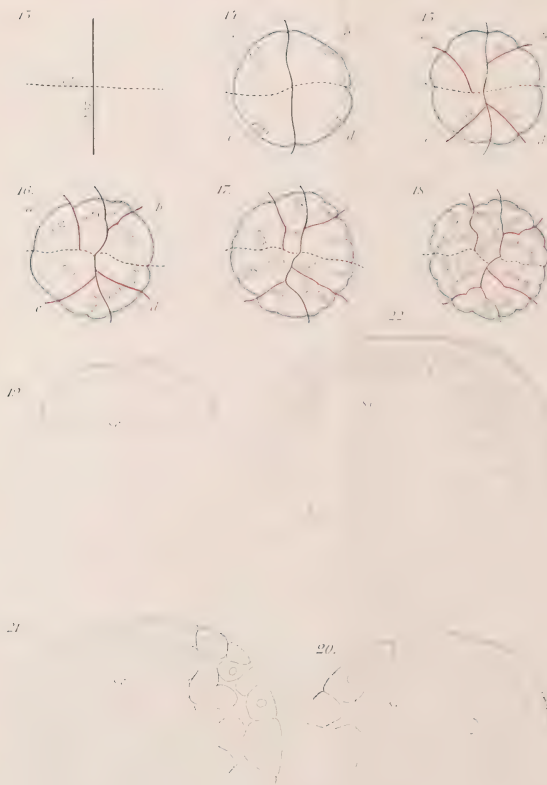


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101.



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EXPLANATION OF PLATE XIX.

FIGS. 1-15. Cleavage in superior hemisphere of egg of *Petromyzon marinus* ($\times 30$).

Figs. 1-4. Successive stages in cleavage of living egg.

Figs. 5, 6, 7. Successive stages in cleavage of second living egg.

Figs. 8, 9. Variations in formation of first and second sets of furrows.

Figs. 10-13. Variations in formation of third set of furrows.

Figs. 14, 15. Later cleavage.

FIGS. 16-35. Cleavage of *Coregonus albus* ($\times 15$).

Figs. 16, 17, 18, 19. Variations in first cleavage.

Figs. 20, 21. Three blastomeres arising from unequal two-cell stage.

Figs. 22, 23, 24. Variations in second cleavage.

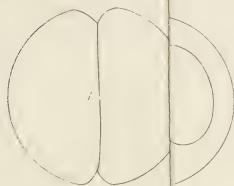
Figs. 25, 26. Conditions derived from unequal two-cell stage.

Figs. 27, 28, 29, 30, 31. Variations in third cleavage.

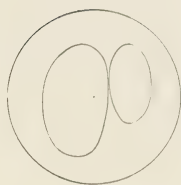
Figs. 32, 33. Fourth cleavage.

Figs. 34, 35. Later cleavage stages.

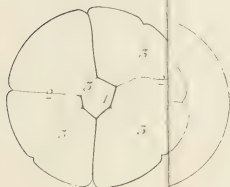
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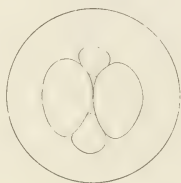
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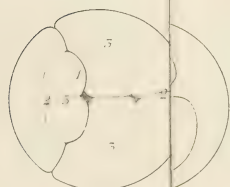
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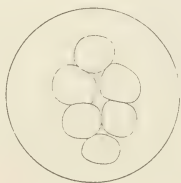
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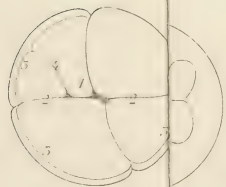
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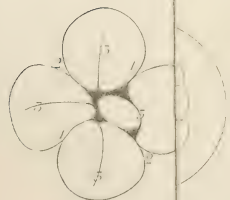
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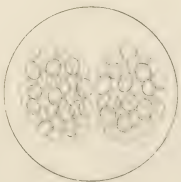
31

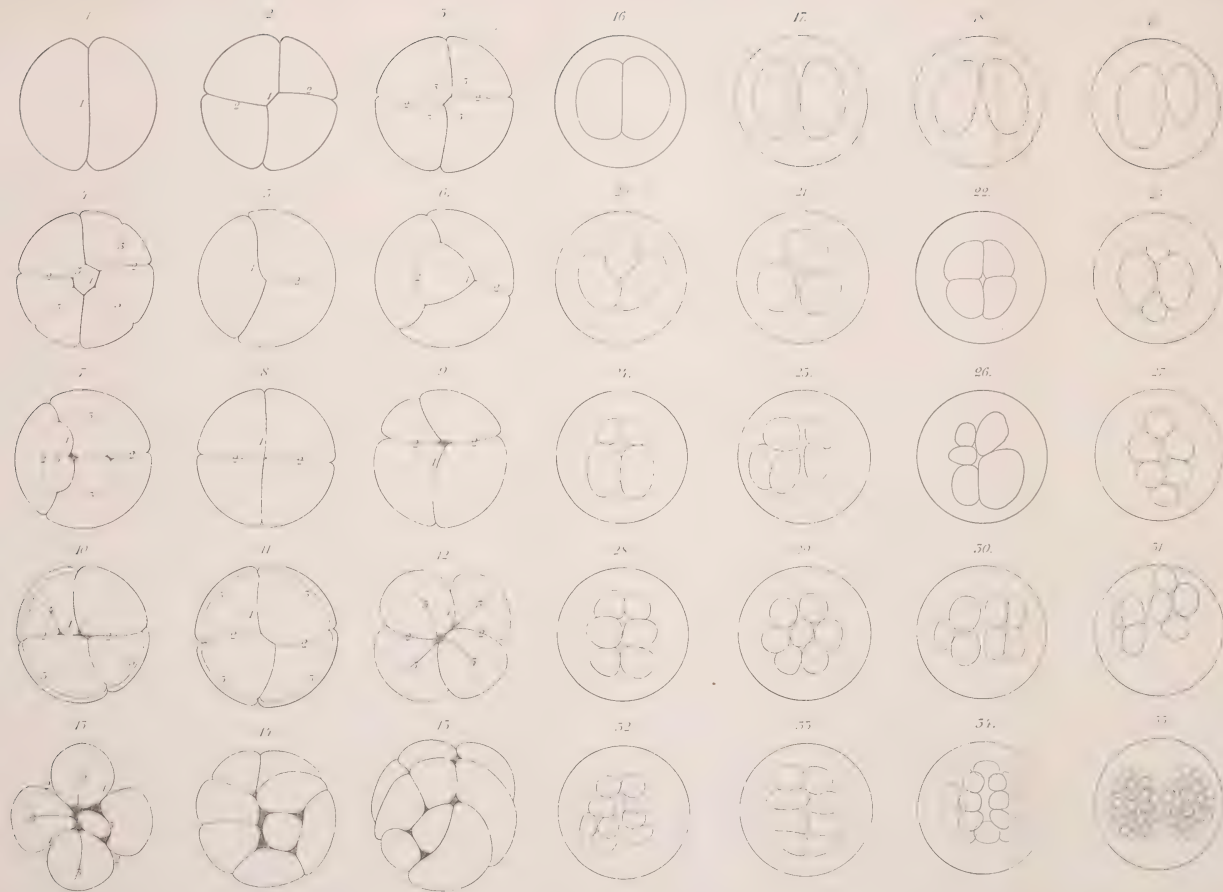


15



35.





EXPLANATION OF PLATE XX.

FIGS. 1-6. Surface views of successive stages in the formation of the embryo of *Amblystoma punctatum* ($\times 15$).

FIG. 1. Surface view, showing irregular depression among yolk cells at vegetative pole, also first indication of blastopore ($\times 15$).

FIGS. 2, 3. Surface views of postero-ventral surface, showing later stages in extension of blastopore ($\times 15$).

FIGS. 4, 5, 6. Surface views of postero-ventral portion, showing successive stages in closure of blastopore, also the formation of neural folds ($\times 15$).

FIG. 7. Ventral surface view of extremely large blastopore ($\times 15$).

FIGS. 8, 9. Surface views of blastopore of *Rana palustris* ($\times 15$).

FIG. 10. Surface view of *Petromyzon marinus*, drawn from living egg by Dr. Johnson, showing lateral extension of the blastopore ($\times 30$).

FIGS. 11-14. Surface views of *Amiurus catus*, showing formation of embryo ($\times 15$).

FIG. 15. Shows portion of germ-ring left behind the embryo.

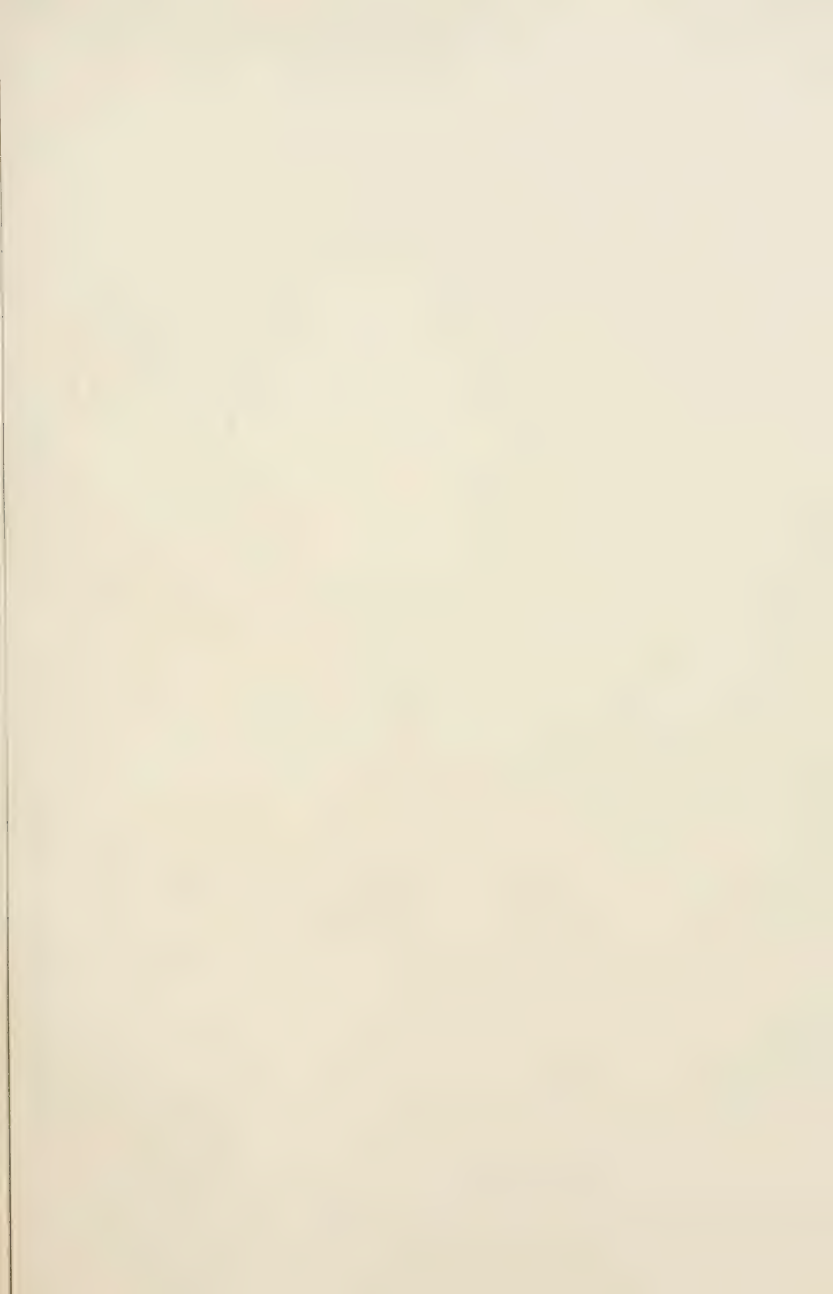
FIG. 16. Surface view of *Coregonus albus*, showing late stage in closure of blastopore ($\times 15$).

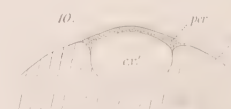
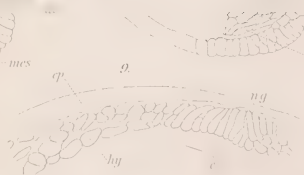
FIGS. 17-24. Successive stages in the formation of the embryo of *Lophius piscatorius*, showing the part of the germ-ring left behind ($\times 45$).



EXPLANATION OF PLATE XXI.

- FIG. 1. Meridional section of egg at stage shown in Fig. 7, Pl. XX ($\times 45$).
FIG. 2. Section along line *xy* of Fig. 2, Pl. XX ($\times 45$).
FIG. 3. Section along line *xy* of Fig. 3, Pl. XX ($\times 45$).
FIG. 4. Saggital section of a stage about the same as indicated in Fig. 4, Pl. XX ($\times 45$).
FIG. 5. Saggital section of egg of *Petromyzon marinus* at a stage indicated by Fig. 10, Pl. XX ($\times 90$).
FIG. 6. Saggital section of gastrula of *Petromyzon marinus*, little later than Fig. 10, Pl. XX.
FIG. 7. Saggital section of *Petromyzon marinus* after Hatta.
FIG. 8. Saggital section of *Petromyzon marinus* showing teloblast.
FIG. 9. Transverse section of *Amblystoma*, at stage intermediate between Figs. 4 and 5, Pl. XX ($\times 90$).
FIG. 10. Section through caudal vesicle of *Lophius* at stage shown in Fig. 24, Pl. XX ($\times 100$).
FIG. 11. Portion of 10 marked (*per.*) more highly magnified.
FIG. 12. Section across post-embryonic portion of germ-ring of *Amiurus* at a stage indicated by Fig. 15, Pl. XX ($\times 300$).
FIG. 13. Saggital section through posterior portion of embryo of *Lophius* at stage indicated by Fig. 21, Pl. XX ($\times 250$).
FIG. 14. Saggital section through posterior portion of embryo of *Lophius* at stage indicated by Fig. 22, Pl. XX ($\times 250$).
FIG. 15. Saggital section through same region at a later stage ($\times 250$).





EXPLANATION OF PLATE XXII.

FIGS. 1-4. Successive phases in closure of neural folds of *Amblystoma* ($\times 15$).

FIGS. 5-8. Successive phases in closure of neural folds of *Rana palustris* showing the infolding of the optic pits, and the formation and extension of the oral and olfactory grooves ($\times 15$).

FIGS. 9-11. Successive phases in closure of neural folds of *Necturus*, showing formation of optic vesicles ($\times 10$).

FIG. 12. Transverse section through dark areas shown in Fig. 5 ($\times 120$).

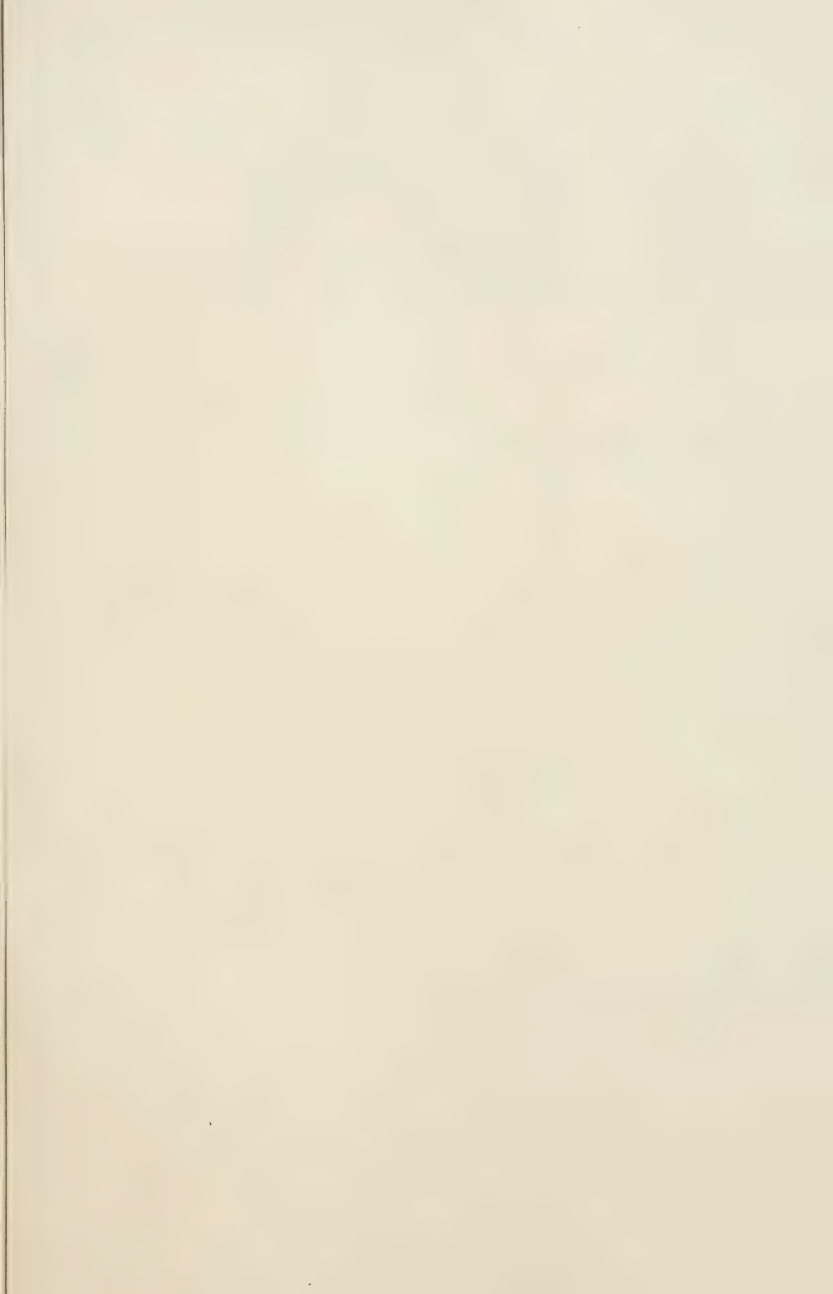
FIG. 13. Transverse section through same areas as shown in Fig. 6 ($\times 130$).

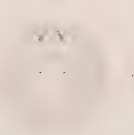
FIG. 14. Transverse section through same areas as shown in Fig. 7 ($\times 100$).

FIG. 15. Transverse section along line 15 in Fig. 10 ($\times 90$).

FIG. 16. Transverse section along line 16 in Fig. 11 ($\times 80$).

FIG. 17. Transverse section along line 17 in Fig. 1 ($\times 100$).





THE FORMATION OF THE FISH EMBRYO.

T. H. MORGAN.

TABLE OF CONTENTS.

| | PAGE |
|--|------|
| NORMAL DEVELOPMENT..... | 419 |
| EXPERIMENTAL OBSERVATIONS: { REMOVAL OF BLASTOMERES..... | 434 |
| { CUTTING THE GERM-RING | 440 |
| ABNORMAL DEVELOPMENT..... | 442 |
| HISTORICAL AND CRITICAL REVIEW | 446 |

THE conception of the formation of the vertebrate embryo by a process of concrescence has gained many adherents since its formulation for the fish by Lereboullet in 1863, and by His in 1874.

Nevertheless, the theory has, from the start, met with no little opposition from other workers who have covered a wide field of investigation.

The solution of the problem in the teleost — the form that gave the starting-point for the theory — seems by no means impracticable, and to involve only a very careful study, both quantitative and qualitative, of the early stages of development. Particularly does the solution seem to be within reach of the experimental method.

During the summer of 1893 I tried the application of this method to the problem. The germ-ring, from which, on the theory of concrescence, the two halves of the embryo arise by a process of apposition, was cut off on one side from its connection with the embryo. Nevertheless, both sides of the embryo that resulted were superficially alike and equal. The obvious conclusion seemed to follow that the germ-ring took no *extensive* part in the formation of the body of the fish.

The conclusion reached is not beyond legitimate criticism, for the question remains unanswered, whether under such con-

ditions the embryo might not have completed itself by methods other than those followed in the normal development. Indeed, in the same communication I showed that, when one of the first two cells was removed, the embryo still formed, although of smaller size.

The present paper is an attempt to meet this possible criticism. In addition, by a detailed examination of the normal phenomena, I have tried to show that my conclusion drawn from the experiment was a legitimate inference.

The processes by which the protoplasmic segmented cap, or blastoderm, of the fish-egg surrounds ultimately the sphere of yolk on which it rests may be first briefly reviewed.

The cap lying at one pole of the yolk-sphere is a convexo-concave disc, circular in outline, with its concave side turned towards the yolk. The cap begins to flatten, and, in consequence, covers a larger surface of the yolk-sphere. The process of flattening and overgrowth continues until the equator is reached, during which time the perimeter of the cap is enlarging. After the equator is passed, the perimeter of the overgrowing blastoderm must steadily diminish in length, while the area covered continually grows larger. When the yolk is covered the perimeter of the disc is reduced to a point and obliterated. The yolk has been overgrown, and the area of the blastoderm has become that of the yolk-sphere enclosed.

Two other phenomena play a conspicuous part during this process of overgrowth. Along one of the meridians of the sphere the embryo forms, with its head resting at the pole around which the blastoderm started. The embryo increases in length along its meridian *pari passu* with the overgrowth of the blastoderm, and its posterior end is always continuous with one portion of the perimeter of the blastoderm.

The perimeter of the cap, or blastoderm, is thicker than the rest of the cap (except in the embryonic portion), and this thickened zone is spoken of as the germ-ring. The germ-ring is a conspicuous feature of the development, and is only obliterated when the overgrowth is completed.

If we examine in detail the method by which the changes sketched above take place, we find them due to a series of

complicated processes that are most difficult to interpret. The first and most important change involves the separation of the three main portions of the blastoderm from the unspecialized cap of cells. The embryonic portion, the germ-ring, and the extra-embryonic area appear.

The cap, as it flattens, shows very early a thicker peripheral ring that gives a strong contrast with the relatively thinner central portion. This ring, almost from the beginning, perhaps even at the beginning, is slightly broader over a portion of its extent, and this gives in the living embryo the first indication of the embryonic side of the disc (Pl. XXIII, Fig. 1). At this region of thickening a tongue of cells pushes up rapidly beneath the surface towards the apex of the disc. Coincident with this event that portion of the cap lying above the tongue becomes thicker, as shown in outline in Pl. XXIII, Fig. 2. This thickening, as my experiments show very conclusively, reaches quite up to the apex of the early blastoderm.

A clearer region of the blastoderm, as seen in surface views, is now left between the embryonic portion and the germ-ring. This extra-embryonic region is broadly semi-lunar in outline, as the figures show (Pl. XXIII, Fig. 2).

The embryonic portion is continuous at its sides with the germ-ring, which has also become more sharply defined during this time.

Serial sections through the blastoderm show to some extent how these changes are brought about. At first the blastoderm is of equal thickness throughout. As it begins to flatten, its center becomes relatively thinner than the periphery. The thicker edge marks the beginning of the germ-ring. In addition to its greater thickness the germ-ring shows a tendency to push certain of its cells under the others, forming in section a small tongue of cells that are continuous with the more developed tongue of the embryonic region.

This process of inturning — whether brought about by an active invagination or as the result of a process of overrolling of the edge I cannot determine definitely — takes place more rapidly and on a somewhat larger scale in the embryonic

portion of the disc. The middle of this region of more rapid growth marks the middle line of the later embryo.

The formation of the embryonic portion is due to two processes. The tongue of cells growing forward beneath the surface contributes to the greater opacity of this region, but the result is more especially due to a relatively greater thickness of the wall over the region where the embryo forms. Here the blastoderm retains almost the same thickness that it had in an earlier stage; while in the extra-embryonic region a rapid process of thinning takes place, until only a single layer of columnar cells is left beneath the outermost layer (Pl. XXIV, Fig. 14, *A*). I have not been able to picture to myself clearly the cell-migrations that bring about or are involved in this process. The phenomenon is a most important one, and I regret exceedingly that I have not mastered the situation.

A few of the changes involved seem to be the following: The area of the blastoderm enlarges somewhat while these changes are taking place, as will be seen by comparing Figs. 1 and 2, Pl. XXIII. The larger surface of the crescentic extra-embryonic region will give room only for the cells already at the surface of that region. These superficial cells will have also increased their surface areas, and will then occupy the larger surface. It seems not improbable that, in addition, some of the lower cells may push up and come to the surface. If, however, all three layers of cells found in this region at the earlier stage (Fig. 1) were to come to the surface, a much greater area would be necessary to accommodate them than is actually present. I therefore infer that from this extra-embryonic region the lower cells must actively migrate beneath the surface to pass on the one hand into the embryonic portion to help to keep it thick while it enlarges, and on the other hand into the germ-ring, producing there a similar result.

In the stage shown in Fig. 1 the thickness of the blastoderm is formed by four vertical layers of cells (excluding the outermost flattened layer), Pl. XXIV, Fig. 13, *C*. When the embryonic region forms, it is at first composed of four vertical layers of cells (with the same exception), and is almost as thick as the early blastoderm of the preceding stage (Fig. 14, *E*). But

since the area of the embryonic region has also increased, it must have received cells from the extra-embryonic region.

The quantitative relations of the *embryo* at different periods of its formation may be next discussed. The series of surface views drawn in Figs. 1-8 give the principal stages of *Ctenolabrus*. The embryonic region is well defined in Fig. 2 (16 hours old), and the head end has extended to the central point of the earlier blastoderm. The head does not now, however, quite reach the center of the present blastoderm. This is due, I believe, to the quicker thinning out of the side of the blastoderm opposite to that side where the embryo is forming, so that the germ-ring is here pushed further away from the earlier center.

In the next figure, Fig. 3, *A*, the broad embryonic region is more sharply marked off from the germ-ring. A side view of the embryo is shown in Fig. 3, *B* (18 hours old).

At the next stage (Figs. 4, *A, B*) the embryo shows a marked axial concentration. In the middle line and anteriorly the embryo is thicker than elsewhere. A little more than half the yolk has been covered by this time (20 hours old).

A side view — optical section — of an embryo 21½ hours old is shown in Fig. 5, *A*. The germ-ring of this embryo is shown in Fig. 5, *B*, in surface view. The posterior end of the embryo is shown in Fig. 5, *C*. These figures show that both an elongation of the embryo and a further axial concentration have taken place. The side view shows that the embryo has also increased in depth (dorso-ventral). The relations that the axial concentration, elongation, and increase in depth bear to one another will be discussed later.

Finally, the form of the embryo when the germ-ring has almost closed is shown in side view in Fig. 6, *A*, in surface view in the three pieces of Fig. 6, *B*, and the posterior-end and germ-ring in Fig. 6, *C* (24 hours old).

Comparing these latter figures with the preceding stage, we see that a marked elongation has taken place. The surface views show, however, that the embryo is nearly the same width in both stages (Figs. 5 and 6), *i.e.*, the axial concentration has about reached its limit. The head-end of the older embryo is even somewhat wider than that of the younger, owing to the

appearance of the eye-evaginations on each side (see Figs. 7, *B*, and 8, *B*). The posterior-end of the younger embryo is much wider than that of the older, and forms in the younger embryo the characteristic tail-knob (Figs. 5, *C*, and 6, *C*). A comparison of the two stages under consideration brings out a most important relation. Before discussing this I should like to refer to other figures of the same stages that have been prepared by another reagent. These figures are shown in Figs. 7, *A*, *B*, *C*, and 8, *A*, *B*, *C*. We see that they have contracted less in hardening than the preceding embryos (osmic). Nevertheless, these (Perenyi's fluid) have retained the same relative proportions that the former showed.

One must conclude from an examination of these stages that, while the breadth (surface) of the two stages is the same, the older embryo has a much less depth and a greater elongation. The conclusion necessarily follows that between these stages *an elongation takes place at the expense of the depth*.

A study of a series of cross-sections at the several stages described above gives additional data as to the method of the formation of the embryo.

A cross-section through the middle of the blastoderm before the thinning out has commenced is shown in Pl. XXIV, Fig. 13, *A*; and a more magnified portion of the same is drawn in Fig. 13, *C*.

A cross-section through the apex of the blastoderm (at 11 A.M.) is shown in Pl. XXIV, Fig. 14, *B*. The apex has at this stage become thinner. A longitudinal median section through the same stage is shown in Fig. 14, *A*. A cross-section through the embryonic portion at this stage shows that it contains a large amount of embryonic material, and that in height it is scarcely less than the average thickness of the preceding stage, Fig. 14, *E*.

A series of three figures through an embryo (at 2.10 P.M., an hour older than that of Fig. 3, *A*) are shown in Figs. 9, *A*, *B*, *C*. Forty sections pass through the embryo, and the first drawn is the seventh of the series. In this figure the embryonic portion is found to have thickened in the axial line. The next figure, *B*, is the 13th section; the third figure, *C*, is the 22d section, and

is at the middle of the embryo. The axial concentration is scarcely seen in this last section. Owing to the greater area of the last section (9, *C*), it contains much more material than either of the preceding.

Three cross-sections through an embryo (at 3 P.M., see Figs. 4, *A, B*) are shown in Figs. 10, *A, B, C*. We find that at this time the axial thickening has progressed more rapidly than in the preceding stage, and extended farther backwards. The first section, *A*, is the 7th of the series; the second is the 20th. This series had 52 sections. The third figure, *C*, is from another embryo, and lies near the center of the embryo. Comparing it with Fig. 9, *C*, we find it thicker, but narrower.

Serial cross-sections through an older embryo (at 5.30 P.M., see Fig. 5, *A*) are drawn in Figs. 11, *A, B*. The first of these is the 18th section. The second is from another series of 116, and is the 85th section, and lies, therefore, 31 sections in front of the posterior end. Great care was taken to get these sections exactly transverse to the long axis. These sections show a much greater axial thickness, and a correspondingly less surface exposure than the last.

The series of cross-sections drawn in Figs. 12, *A, B, C, D*, come from embryos (at 7.05 P.M., see Fig. 6, *A*). The first of these, *A*, is the 26th, the second, *B*, is the 32d. Figures *C* and *D* are from another embryo. Of these; *C* is the 35th section anterior to the posterior end, and *D* the 13th in front of the posterior end.

Comparing the last two sets of figures, Figs. 11 and 12, we find that, while the surface (dorsal) exposure is about the same in both sets, *the depth of the younger series is greater* than that of the older. This is true, both for the anterior and posterior parts of the body, and is seen most strikingly when more enlarged figures, of the two stages, are compared. This result agrees with that noticed in surface preparations and optical sections.

A volumetric comparison of all of these sections from corresponding regions of the different embryos would be most valuable, because we could then determine whether all of the area of the younger and wider sections was greater, or equal, or less, than

the corresponding section of older embryos. This would give data to determine whether the elongation of the embryo was due solely to axial concentration of the earlier embryonic region, or due to other processes. For, obviously, if the areas of the older sections were less than the areas of the corresponding younger sections, an axial elongation must have occurred to accommodate the mass. Practically, the difficulties in the way of such comparisons are very great, and the chances of error very large. I have, therefore, not attempted to make such comparisons in detail. The only safe method by which to get at the exact data will come, I think, from accurate reconstructions of such embryos. Then, the relation by weight between the various parts and sections can be readily made.

A study of all of these sections shows nevertheless most clearly one important result. The areas of the sections through the posterior end of the embryonic shield of the earlier stages, are much greater than the areas of those sections of older embryos, taken at the same absolute distance from the head. It follows that all of the material of the posterior part of the younger stages is not needed to form the same portion (*i.e.*, the portion at the same distance from the anterior end,) of the older embryo. Two processes must then take place simultaneously in the embryo as it elongates posteriorly: 1st, an axial concentration; 2d, *a transfer backwards of a large part of the material.*

In the older stages a third process takes place, to assist in the elongation, *viz.*, an axial elongation of the whole embryo. The embryo elongates as the cells press in toward the central longitudinal axis. (See stages shown in Figs. 11 and 12.)

We may next turn our attention to a study of the germ-ring. From a theoretical point of view there are three phases in the growth of the germ-ring: 1st, a period of formation out of the blastoderm; 2d, the growth of the germ-ring to the equator of the egg — a period of continuous increase in its length; 3d, the period of growth from the equator to the closure, and disappearance of the ring. During the last period it is continually getting shorter. We see from the surface that the breadth of

the germ-ring, during its overgrowth, goes through a series of changes. When it is first established, its breadth is shown in Fig. 1, *A*, and Fig. 2, *A*. When it has extended some distance over the yolk, as shown in Fig. 3, *B*, we find that it has diminished a little in breadth. When the germ-ring has just covered the equator of the egg, as shown in Fig. 4, *A*, we find that its breadth has very much diminished. An examination of stages intermediate between the last two, shows the process to be due to a continuous and gradual change. We see then clearly that while the germ-ring is increasing in length it decreases in breadth — just as an elastic band would change if drawn over a sphere. Moreover, if we artificially remove a part of the yolk during these stages, we find that the germ-ring is highly elastic, and contracts at once to fit the smaller sphere.

After the equator is passed we find that the germ-ring slowly increases in superficial breadth again, as Fig. 5, *B*, and Fig. 6, *C*, show. Again we find a very exact resemblance to an elastic band, under similar circumstances.

Sections cut exactly at right angles to the length of the band are most instructive. The section drawn in Pl. XXIV, Fig. 13, *B*, corresponds to one side of the whole section shown in Fig. 13, *A*. The edge of the blastoderm is somewhat thickened, and the cells show a tendency to turn under.

The next figures are taken from an embryo at 11 A.M. (Fig. 2, *A*). Figs. 14, *A* and *B*, show respectively longitudinal and cross sections through the blastoderm, and *C* and *D*, more magnified portions of the germ-ring. At this stage the center of the blastoderm has become thinned out, leaving the edges of the blastoderm, that form the germ-ring, in the condition shown in the figures. We find that the germ-ring, both in front (*C*) and at the sides (*D*), is composed of a large number of cells, and has a very considerable breadth. We find, moreover, that the cells of the upper layer (beneath the 'covering-layer') gradually pass into the cells of the extra-embryonic region. The most important point that such figures show, is that the *anterior* portion of the germ-ring has less breadth, and is composed of a fewer number of cells than the *sides* of the

germ-ring. This difference is present, so far as I have been able to determine, from the very beginning, and persists, as will be shown through all the later history of the germ-ring.

Sections, both longitudinal and cross, through the embryo and germ-ring, at 1.10 P.M., are shown in Figs. 15, *A*, *B*, *C*, *D*. The first section, *A*, passes through the longitudinal median plane of the embryo, and shows the extra-embryonic region and germ-ring in front of the embryo. Fig. *B* passes transversely through the apex of the extra-embryonic region, cutting the germ-ring at the two sides. More magnified portions of the germ-ring of these two sections are shown in *C* and *D*. We find that the number of cells in the germ-ring has greatly decreased, and that now only two layers are evident in section — the outer continuous into the extra-embryonic region, and the inner tongue of cells beneath. Occasionally, a few cells are found between these two layers. We find that the germ-ring is narrower, and the cells of its upper layer are not so numerous as in the last stage.

The under layer of cells forming the tongue has about the same extent as in the preceding stage, but has fewer cells. Comparing Figs. *C* and *D*, we see at once a decided difference in size of the two. The lateral section, *D*, contains more cells and a longer tongue of inner cells, and the cells themselves are higher.

Three sections are drawn in Figs. 16, *A*, *B*, *C*, from an embryo killed at 3 P.M. (Fig. 4, *A*). The germ-ring in section is smaller than in the last figures, and contains fewer cells. If we compare the amount of material in the germ-ring at this stage with the amount of material in the embryo, we are struck at once with the comparatively small amount in the germ-ring. The embryo is not yet two-thirds its full length and we see how inadequate the material of the germ-ring would be to complete the remaining third.

Sections through the germ-ring of an embryo at 5.30 P.M. (Fig. 5, *A*) are shown in Figs. 17, *A*, *B*, *C*. The first of these passes through the open yolk exposure, and the germ-ring is cut on each side. Fig. *B* is a more magnified portion of the same, and shows how extremely thin the germ-ring has

become. Fig. *C* cuts the germ-ring very near to the embryo, and here we find quite an accumulation of material — relatively a very large amount.

Finally, in Figs. 18, *A*, *B*, we have drawn to the same scale as the preceding figures, sections through the germ-ring of embryos killed at 7.05 P.M. (Fig. 6, *A*). The first section is from a median longitudinal section of the embryo, and cuts the germ-ring at a point exactly opposite to the embryo. The germ-ring of this figure (*A*) should be compared with the germ-ring of Fig. 14, *C*, through the corresponding region. Fig. 18, *B*, is from a cross-section of the germ-ring at the side (not near to the embryo). These sections show us to what a small structure, quantitatively, the germ-ring has been reduced during overgrowth of the yolk, and what a few cells it contains after the process. The apparent meaning of this change will be more fully discussed below.

From a series of surface preparations of the extra-embryonic region, I have attempted to discover what part the cells covering this region play in the process of overgrowth.

I hoped that such a study might give some clue as to the part taken by the germ-ring in the process. If the number of cells over this region increased in number at a greater rate than their own processes of cell-division would account for, then the new cells added must come in from the germ-ring. If, on the other hand, the number of these cells remained constant throughout, then the germ-ring could take no part in the formation of the extra-embryonic region, and the germ-ring must ultimately pass entirely into the embryo. I have worked on material prepared by osmic acid (followed by Merkel), but these preparations have not given sufficient data in regard to karyokinetic processes, that may go on in the extra-embryonic region. It is important to determine the latter point, and I regret that my material has not been adequate to do it. (See Appendix.)

In the following figures the outlines of the cells figured correspond to the second layer of surface cells of the earlier stage, since the outer, very much flattened layer (covering-layer) of the earlier stages becomes very thin during the later stages,

and the cell-outlines are not apparent in the osmic preparations. (See Appendix.)

The series of eggs from which the first three figures were taken had been fertilized at 10.30 A.M. The first lot were killed at 6.05 P.M. and are, therefore, $7\frac{1}{2}$ hrs. old. The surface cells of the *inner* layer of the extra-embryonic region of this stage are shown in Fig. 19, *A*. The group of cells *A* is taken from that portion of the blastoderm that is to become the extra-embryonic region. The group marked 19, *B*, comes from that portion of the surface where the embryo will subsequently form. Even at this early stage the difference in size of the cells of the two regions of the blastoderm is very marked. I have not made any attempt to see how far back this difference can be found in the earlier stages. Henneguy says that Kowalewsky found it quite early, and I have seen that it is present before any other structures indicate where the embryo will form.

The next two figures, 20, *A*, *B*, are taken from a blastoderm at 8.30 P.M., from regions corresponding to those of the last figures. The contrast between the two sorts of cells is now much more marked than in the preceding stages, because the extra-embryonic cells are larger than before, and the embryonic cells are smaller.

The next figures, 21, *A*, *B*, are from an embryo at 9.30 P.M., and the contrast is found to be more marked than in the last, and for the same reasons. At 19 hours after fertilization the cells of the extra-embryonic region are not very much larger than in the last, but the superficial embryonic-cells are decidedly smaller (Fig. 22). At 20 hours the two sorts of cells are shown in Figs. 23, *A*, *B*. In *C* a few cells are shown from the posterior end of the embryo above the 'tail-knob,' and in *D* the superficial cells of the second layer of the germ-ring are drawn. The cells of the germ-ring are intermediate in size, between those of the extra-embryonic region and the embryo. Those in the posterior end of the embryo (*C*) are larger than those in the anterior end (*B*). This same relation seems to hold throughout the series.

At 24 hours when the germ-ring is nearly closed, the size of the extra-embryonic cells is shown in Fig. 24. At this stage

the embryonic cells are too small and too irregular to make it worth while to draw them.

The main conclusion that must be drawn from these figures is, that as the extra-embryonic area increases in extent, there is also an increase in the area of each cell of which it is formed. A corresponding flattening of these cells takes place at the same time, so that when the yolk is finally overgrown an exceedingly thin membrane is left to cover the extra-embryonic region, germ-ring, and embryo.

The main question to be decided is this: Will the enlargement of the individual cells be sufficient when all taken together to account for the greater enlargement at each stage of the extra-embryonic region? We must also determine whether the number of cells in this area is the same throughout or becomes greater. In order to get an answer to these questions with as small a chance of error as possible, I made spheres of clay, as much larger than the real fish-egg as the cells themselves had been magnified. In the spheres (magnified 370 times) cells or groups of cells were traced and then a count made of the result. Three stages were selected, one corresponding to an embryo at 11 A.M. (Fig. 2, *A*), one at 3 P.M. (Fig. 4, *A*), and one at 7.05 P.M. (Fig. 6, *A*). In each case the embryonic area and the germ-ring were also sketched on the enlarged egg, and their areas excluded from the part covered by the extra-embryonic cells.

The embryos at 11 A.M. were found to have 1785 cells in the extra-embryonic region. Both sphere and cells were magnified 370 times, and the superficial area of the extra-embryonic region was equal to 125 square centimeters. A square of 5 centimeters on each side (= 25 sq. cm.) contained 357 cells.

At 3 P.M. the number of cells was found to be 4494 in the extra-embryonic region. There were about 1050 square centimeters, each 25 containing 107 cells.

At 7.05 P.M. there were 6040 cells in the extra-embryonic region. There were 2000 square centimeters and each 25 contained 76 cells.

These results show that, as the area of the extra-embryonic portion increases, the size of each cell increases and the number

of cells also becomes larger. When the yolk-sphere is covered there are nearly three and a half times as many cells in the extra-embryonic region as in the first stage. What is the source of these new cells? They may have come from the germ-ring or they may have come by division of the cells themselves. There is direct evidence, as we have seen, pointing to their origin in part from the germ-ring. On the other hand, if the cells had divided very rapidly during overgrowth they would have become smaller. Cell growth, or simply superficial flattening, might, however, compensate for the reduction in size of the cells if cell-division takes place continuously. Therefore, without a knowledge of how extensively cell-division is taking place we cannot decide this problem. (See Appendix.)

The bearing of results recorded in the preceding sections may be summed up as follows:

The most important period in the formation of the embryo seems to be at the time when the embryonic portion (head) is first formed. If it could be shown that the amount of material present at that period in the embryo is equal to the material of the fully formed embryo, there would remain no doubt that the elongation of the embryo was due to axial concentration. In the absence of data we must turn to other sources for information. A study of the method of the formation of the embryo has shown us that the axial concentration must necessarily contribute to the elongation of the embryo backwards. A study of the germ-ring points decidedly to the conclusion that one of its functions, at least, is to contribute cells to the extra-embryonic region. Another possible function of the germ-ring is that it may continually bring new cells into the posterior end of the elongating embryo. According to His' conception of concrescence, the sides of the embryo are laid down right and left by the *apposition* of the germ-ring, so that new material is constantly coming up to form the posterior end of the embryo. We have seen, however, that during the first period of the overgrowth of the germ-ring it is constantly increasing in length, and correspondingly decreasing in breadth. After the equator is passed the reverse process takes place. And during both of these periods there is strong evidence

pointing to the loss of some cells from the germ-ring, and to their conversion into cells of the extra-embryonic region.

There is direct evidence to show that the germ-ring, after it has passed the equator, is entirely inadequate to contribute any large amount of material to the embryo. The sides of the embryo are then far too great, in proportion to the germ-ring, to allow any such formation as His supposed. It must be remembered, too, that in these pelagic fish eggs, the embryo elongates posteriorly *pari passu* with the overgrowth of the germ-ring. We have seen that during overgrowth both upper and under layers of the germ-ring have decreased in quantity, and in the number of their cells. We can account for a part of this loss in the uppermost layer, by the continuous addition it seems to make to the extra-embryonic region. We cannot account in a similar way for the loss of the cells of the under layer (meso-endoderm). We have seen that there is always a larger accumulation of these under-layer cells near to the embryo, and the number of cells is less and less as we pass outwards along the germ-ring. This strongly suggests that the cells are passing continuously from the germ-ring into the embryo. This must certainly be the case after the equator of the egg has been passed by the germ-ring, and sections show that it is in these latter stages of overgrowth that the contrast between the different portions of the germ-ring is greatest.

Whether or not the same thing happens before the equator is reached, is not so easily determined, because during this period the germ-ring itself is getting longer, and the increased length might account for the decrease in the number of cells of the inner layer in each cross-section. But during these early stages the sides of the embryo are not so sharply marked off from the germ-ring, and there is direct evidence to show that this lateral material passes into the embryo along each side of the middle line, while the tail-knob grows backwards along the axial mid-line.

I therefore conclude that during the elongation of the embryo, material is continuously passing in from the germ-ring, and is laid down with the axial material already present.

Further, the great disproportion in the later stages between the whole amount of material in the germ-ring, and the amount of material necessary to form the embryo, makes it quite certain that during the late stages only a *relatively* small amount passes into the embryo from the germ-ring.

During the second period of overgrowth of the germ-ring we have seen from surface preparations that the germ-ring gets broader as it begins to close. Sections show us, however, that this does not mean that it is quantitatively greater, but the reverse. Everything points to the conclusion that during this period, while the germ-ring is shrinking, it is also losing cells to the extra-embryonic region. Its own cells become flatter, and surface preparations produce the effect of lines of cells running out from the germ-ring into the extra-embryonic region. The latter effect may, however, be due to the shrinking of the ring. The line of demarcation at this time between germ-ring and extra-embryonic region is, in preparations, not at all a sharp one, as the figures might seem to indicate, but we find the germ-ring gradually fading out into the extra-embryonic region.

If it is admitted that the under layer of the germ-ring passes continuously into the embryo, it follows with a good deal of probability that the upper (second layer) does also. First, because of its close connection at the edge of the blastopore with the under layer, and secondly, from the evidence furnished by embryos whose development has been modified by artificial means. These embryos will be described in the next section.

EXPERIMENTAL.

REMOVAL OF BLASTOMERES.

In my preliminary paper published in the *Anatomischer Anzeiger*, 1893, I have described the results of certain experiments on the eggs of *Fundulus*. It was found possible to remove from the egg one of the first two blastomeres without destroying the capacity of the remaining blastomere to develop. The blastomere, at first flattened on one side where it had been in

contact with the blastomere removed, rounds up and continues to segment. Only those cases were described where one blastomere had been *completely* removed by the operation.

Records of sixty-eight cases were obtained, and in these the early stages of segmentation were followed and embryos reared. About twenty of this number died, however, before the embryo appeared.

If we take, as an example, an egg in which the first two blastomeres are equal in size, and remove one of the two cells, we find that, after rounding up, the remaining blastomere goes into a resting stage. The egg operated upon then falls behind the normal egg in the rapidity of its divisions.

When the blastomere divides, it does so into two equal or nearly equal parts (25, *B*),¹ and the result is in all respects except size a copy of the normal two-cell stage. The furrow of this first division appears always in the plane where the second furrow of the normal egg would lie.

The second furrows succeed the first and at right angles to it; and a four-cell stage results like the normal stage of corresponding segments, except in point of size (25, *C*).

The third furrows come in more or less at right angles to the last, but the regularity from this time on is lost (25, *D*); and I have not found comparisons between the small egg and the whole egg in these later stages particularly profitable. Another more regular eight-cell stage ($\frac{1}{2}$ -16) is shown in Fig. 26, taken from another series where the *first* normal cleavage was equal.

In order to detect possible errors in observations made on the living eggs, a number were hardened as soon as one of the first two blastomeres had been removed. Other eggs operated upon were hardened at the two, four, eight, and many-celled stages. These eggs were then cut into serial sections and examined. They showed in every case that the nucleus of one blastomere had been removed with the protoplasm, and only the nucleus (and protoplasm) of the other cell (or its products) remained.

¹ In Fig. 25, *A*, the first cleavage gave two unequal cells. The smaller of these was removed, and from the larger the series 25, *B*, *C*, *D*, developed.

The sections show, moreover, a condition that had not been appreciated in surface study. A certain amount of protoplasm had collected beneath the cells derived from the blastomere that had been left. This protoplasm must belong to the blastomere removed. It is a well-established fact of teleostean development that protoplasm from the surface of the yolk-sphere continues to flow up into the blastoderm during the early stages of cleavage. Apparently, this process continues in the egg operated upon, although there are no cells on one side to receive the protoplasm flowing up into this region. This I believe to be the origin of the protoplasm that was found accumulated beneath and to one side of the half-sized blastoderm.

In my former contribution I stated that the protoplasm from the yolk-sphere that continued to flow into the blastoderm went, in all probability, entirely into the cells that remained. I said: "Presumably the same process takes place in the egg operated upon, so that the half blastomere increases in size by the protoplasm that it would receive had it remained in connection with the one removed; but also must receive the additional protoplasm that would normally have gone into the other removed blastomere. Hence a blastoderm larger than half is formed, and from this an embryo larger than half an embryo." This assumption, my sections show, is not altogether true, although indirectly the same result is brought about at a later stage.

My statement that "the size of the embryo is determined by the amount of protoplasm present and not by the quantity of nuclear matter" is, however, sufficiently demonstrated, I believe, by the following experiment. "Often the first cleavage-plane of the normal egg divides the blastodisc into very unequal parts (Fig. 25, *A*).¹ In some cases the larger blastomere has been removed; in others the smaller (Fig. 25, *A*). The result is the same in either case, as a perfect embryo is formed; but the embryo is smaller when the smaller blastomere is left, and larger when the larger blastomere remains."

¹ In Fig. 27 is drawn the four-cell stage of a normal egg, in which the first division had been very unequal, more so than that of Fig. 25, *A*. This egg was isolated and a perfect embryo developed from it.

I have studied in serial sections many of the embryos that have developed from those eggs where one blastomere had been removed. The principal problems were first to determine whether such embryos were composed of the same number of cells that make up the normal embryo; and secondly to see whether these cells and their nuclei were of the normal size.

An embryo that has developed from an egg operated upon is shown in Fig. 28. The normal embryo of corresponding age is drawn in Fig. 29. The first is drawn as seen from above; the second (normal) as seen in side view. Surface views of the embryo showed that it is shorter and narrower from side to side than the normal embryo. The cross-sections of this embryo were not very satisfactory; yet they showed two important points. *On one side*, particularly in the posterior region of the embryo, was found a layer of peculiar cells lying between the ectoderm and the parablant. These cells were larger than any other cells of the embryo, and very different in structure. Their nuclei were very indistinct, their protoplasm loose, and the cells rounded, *i.e.*, not flattened to any extent against one another. There is every reason to believe that these cells have come from the protoplasm that belonged to the cell removed. The nuclei have come in all probability from the nuclei of the embryo. The protoplasm has been "post-generated," and one will recall in this connection the description given by Roux of the regeneration of the injured side of the frog's egg from wandering nuclei (cells?) from the living half.

Another point that these sections showed is that the size of the nuclei of the "half-sized" embryo is not appreciably smaller than those of the normal embryo of the same stage.

A more detailed comparison between the normal eggs and eggs operated upon I have been able to make for older embryos at the time when the germ-ring has just closed.

Surface preparations show that in all cases the *length* of the embryo coming from the eggs in which one blastomere had been removed is less than normal. There is great individual variation, however, in this respect. Three figures of embryos are shown in Figs. 30, *A*, *B*, *C*. The first of these, Fig. *A*, is

a side view of a normal embryo; Fig. *B* is from an egg operated upon and the largest embryo of which I have a record; while Fig. *C* is the smallest embryo of the lot. Generally we find that the embryo from the egg operated upon lacks about one-fourth the normal length.

The breadth of these embryos is always a little less than the normal. This is most obvious in the head after the formation of the optic vesicles. There is here, also, much variation, as shown in the three heads drawn in Figs. 31, *A*, *B*, *C*. The first of these is the normal (*A*), and the other two come from eggs operated upon. The figures are from preserved material.

The depth (dorso-ventral) of these embryos is generally less than that of the normal, but here, again, there is a great deal of variation.

These embryos were cut into cross-sections. The cross-sections of the shorter embryos are, in nearly all cases, smaller than the cross-sections of the normal, but the extent of this will depend much on the individual.

Careful camera drawings of the nuclei of the different organs of the body have been taken and comparisons made between the nuclei of the normal eggs and eggs operated upon. The result is most unexpected. *There is no difference in the size of the nuclei of the two sorts of embryos.* This holds throughout for nervous system, eye-vesicles, mesoderm and entoderm. When we recall that these nuclei have come from only one-half of the original segmentation nucleus, it is remarkable that at this late stage of development there should be no difference in size of nuclei between the normal and operated embryos.

Another equally unexpected result was found. If the nuclei in the cross-sections passing through corresponding regions be counted, we find the number practically the same in both normal and operated embryos. As an example of this I may cite one case. In a cross-section of a normal embryo passing through the eye-vesicles I found 117 nuclei in the central nerve-chord and notochord. In one eye-vesicle in section there were 67 nuclei, and in the other 65. These numbers are not perfectly accurate, but are the average of several counts, ranged

within a difference of ten for the most extreme cases, generally much nearer together.

In a cross-section of an embryo, developed from one of the first two blastomeres, passing through the eye-vesicles I found 124 nuclei in the central nervecord and notochord. In one eye-vesicle there were 70 nuclei; in the other, 66.

The results from the two cases are very close, and, although there is some chance of error in the counting, yet there can be no doubt that the number of nuclei is about the same.

The absolute number of nuclei in the smaller embryos must be, nevertheless, smaller than in the normal on account of the difference in length of the two embryos.

The results show, I think, that the smaller embryo, instead of elongation by axial concentration to the full normal length, tends to form an embryo whose cross-section is not greatly different from that of the normal. That is to say, elongation takes place to a less degree where there is less material to expend, and most of the material is utilized to form an embryo approaching the normal, as far as possible, for the *part formed*.

The number of *cells* in the cross-section cannot be so satisfactorily counted. Their number must correspond to that of the nuclei present; hence, since the number of nuclei is the same in the two cases, the number of cells must be also the same. Since the embryos from one of the first two blastomeres are smaller than the normal, it would seem that the size of each cell in the former must be a little less than in the normal. I do not place much confidence in the last deduction, for it leaves out of account the intercellular spaces, and the chance of error from this source is too great to allow any special weight to be attached to the conclusion regarding the size of each cell. Actual measurements do not show any decided difference in the two cases.

The conclusions given above are not the result of a comparison of a few sections, but are the abstract of a large number of observations. Since they all agreed, it did not seem worth while to dwell at greater length on the subject. The fact that the protoplasm that accumulates beneath the embryo, and which belongs to the removed blastomere, and therefore is at

first non-nucleated, — the fact that this subsequently becomes nucleated and goes into the mesoderm at the side of the posterior end of the body, is of special interest, and needs to be especially emphasized. It is probably in part due to this that an embryo larger than half is formed from one of the first two blastomeres when the first two blastomeres are of equal size.

CUTTING THE GERM-RING.

In my preliminary communication I have described a series of experiments, by means of which I believe that it has been shown, experimentally, that as soon as the head is definitely established it remains a fixed point, and the elongation of the embryo takes place posteriorly. When the germ-ring first appears it is thickened along one side. On this side the head rapidly forms, reaching from the thickened germ-ring to the apex of the early blastoderm, *and formed largely from material that has never been at the edge of the blastoderm.*

In another set of experiments I attempted to cut the germ-ring to one side of the embryo. 'In a few cases in which the germ-ring was cut at one side of the embryo, with a hot needle, the embryo continued to develop, although the germ-ring remained intact only on the uninjured side. In several cases, out of a very large number, where the germ-ring was cut in two by a sharp cold needle, the cut ends drew apart and did not unite again.' From these experiments I drew the conclusion: The embryo, cut off from all connection with the germ-ring on one side, elongates backwards, producing an embryo, having both right and left sides alike and equal. In the elongation of the embryonic knob backwards, the head remains a fixed point, and the elongation is due to an extension backwards of the mass; the germ-ring, therefore, takes *no important part* in the formation of the body of the fish-embryo.

In support of these conclusions I now figure two of these embryos. In Pl. III, Figs. 39, *A*, *B*, *C*, we find the germ-ring cut in *A*, at the time when the head has just been established. The embryo was killed when the germ-ring had not yet closed, as seen in *B*. We see in this figure that the

right posterior end of the embryo is completely cut off from the germ-ring of that side. A cleft in the extra-embryonic area runs out from the blastopore. It will be seen that the germ-ring, cut off from the embryo on one side, has never again reunited, but nevertheless a perfect embryo has formed, as drawn in *C*.

Cross-sections through this embryo show that the mesoderm on the injured side of the embryo, in the region of the tail-knob, is almost absent. Farther forward there is less mesoderm on the injured side than on the normal side, but in the middle and anterior part of the embryo the same quantity of mesoderm is present on both sides.

At the posterior end of the body the ectoderm has not turned in so perfectly to form the central nervous system, and it is due to the presence of lateral ectoderm that, in the absence of the proper amount of mesoderm, the external symmetry *seems* to be perfectly preserved.

The result of another experiment is shown in Figs. 40, *A*, *B*. Here the germ-ring was cut at the side of the embryo before the complete establishment of the head. The embryo was killed at a stage when it had still a large blastopore (*B*). To the left of this embryo a line of dead cells (or abnormal cells) marks the line where the earlier severance of the germ-ring took place. It will be seen that an imperfect union has taken place between the germ-ring and the embryo along the line of necrotic cells. With this exception, the embryo is normal. To the outer side of these necrotic cells there is a thickened mass of cells continuous into the germ-ring.

I should interpret this result to mean that after the severance of the germ-ring the head established itself, and the posterior extension of the embryo followed. Owing to the imperfect reunion of the germ-ring with the embryo, an accumulation of cells has taken place on the outer edge of this barrier. It looks as though this mass of cells belonged to the embryo, but was prevented from passing into it by the hindrance due to the wound. If this interpretation be correct, two conclusions follow that are of importance in forming any conception of the relation of the germ-ring and embryo in the Teleost:

1. The embryo can elongate posteriorly without the addition of material from the germ-ring.

2. Under normal conditions a certain amount of material passes from the germ-ring into the embryo.

Another interpretation of the results of such an experiment suggested itself early in the work. If one-half of the early two-cell stage may develop a perfect embryo, why may not the embryo cut off from the germ-ring also reproduce the rest of the body on the injured side? An answer was not far to seek. The early stages of development are passed through quite rapidly, and if one-half of the embryo were regenerating it would certainly lag behind the normal half, and the embryo would bend over towards the regenerating side. But a study of the embryo shows that there is very little, if any, delay in the elongation of the embryo. The tail-knob and neuro-noto-chordal plate elongate symmetrically, and only the mesoderm is unsymmetrically developed. These facts, taken in connection with a study of the normal processes, exclude any interpretation of the phenomenon as involving regeneration.

ABNORMAL DEVELOPMENT.

Attempts were made to modify the development of the embryo, by placing the developing eggs of *Ctenolabrus* into artificial media. The addition of more salt to the sea-water (1 to 5 parts to 100 parts sea-water) gave no results of interest. Many embryos developed normally in the more dilute solution (1 part NaCl), but nearly all died in the more concentrated solution (5 parts). The eggs had been put into the solutions when the embryo had just appeared. Another lot were placed in the solution when the embryo had reached the equator of the eggs, but the same results followed.

The addition of alcohol to the extent of 1% to the sea-water, did not seem to seriously affect the development of the embryos. The addition of $2\frac{1}{2}\%$ of alcohol delayed the closure of the blastopore in many of the eggs, and killed some outright. Stronger solutions of alcohol (5% and 10%) killed the embryos very quickly.

The most successful results were obtained by adding fresh water (from a well) to the sea-water. When put into a solution containing 50 parts of sea-water and 25 parts of fresh water, many embryos developed normally, others showed that the closure of the blastopore was delayed, and several abnormal forms were obtained. The best results were from a mixture of half fresh water and half sea-water. The development of many of the embryos was altered, and generally in a peculiar way. Most of the eggs were killed however by the solution. Eggs put into this solution at the 64-cell stage were dead after 24 hours. Other eggs were put into the same solution when the germ-ring had just appeared. Very few formed normal embryos. Many died. Several formed abnormalities. Some of these abnormal forms are shown in the following drawings.

One very common modification is shown in Figs. 31 and 32. These figures show that the solution has retarded very much the elongation of the embryo posteriorly, but it has not correspondingly affected the overgrowth of the germ-ring. Both figures are from hardened embryos at a time when the yolk is still exposed (*y*) to a small extent. When, finally, the germ-ring closes, the embryo is much shorter than the normal, and much broader. Both the figures show that the embryo is much broader than any stage of the normal embryo. The central plate that should form the nervous system and notochord is very much flattened out, and the periphery of this plate is bordered by a zone of tissue which is directly continuous into the germ-ring.

Cross-sections made through this embryo (Fig. 41) show that the germ-ring is directly continuous into the ring around the embryo, and that the quantity and quality of the material seems to be the same in each in cross-section. The border, or ring of the embryo, is composed largely of mesodermal tissue continuous with the lower layer of the germ-ring, and one cannot but draw the conclusion that the material is the same in each.

The central plate of the embryo is found to be composed of rounded cells, loosely held together, and the plate is vertically much thinner than the border zone of mesoderm. The tissue

in the germ-ring and lateral ring of the embryo is formed of polygonal cells that appear normal.

Another embryo is drawn in Fig. 33. Here the elongation of the embryo, and the closure of the blastopore, has taken place more normally than in the last cases. This embryo also shows, with great distinctness, that on each side of the neuro-notochordal plate, a band of tissue is directly continuous into the germ-ring, and we see this tissue is also distinctly separated from the central neuro-notochordal plate.

It is not at all uncommon to find embryos that show the condition seen in Fig. 35. The tail-knob projects beyond the meeting point of the germ-ring, *i.e.*, the tail-knob projects into the blastopore region.

Another such modification is shown in Fig. 36, where the protrusion of the tail-knob is greater than in the last. The germ-ring is attached further up on the left side of the embryo than on the right. This embryo was cut into serial cross-sections. The sections show that the ectoderm destined to form the nervous system has not concentrated along the axial line but is spread out in a broad, flat plate over the posterior dorsal surface of the embryo. In the middle line, beneath the flat plate, the rounded notochord is perfectly formed. The mesoderm forms two thick bands along each side of the notochord, and is composed of polygonal cells.

The sections through the posterior end of this embryo show that the lateral sheets of mesoderm continue into the under layer of the germ-ring, and it seems, moreover, that it is only the outermost portions of the mesoderm of the sides that go into the germ-ring, so that a small amount continues, for a short distance along the sides, posterior to the point of union of the germ-bands. It looks, from the evidence furnished by these sections, as though the mesoderm of the germ-ring laid itself down along the sides of the mesoderm of the body.

The protrusion beyond the level of the union of the germ-bands is the tail-knob, in which the three (?) layers are not sharply separated from one another.

Another point of interest is seen in this series of sections. The ectoderm above the germ-ring near to the embryo is

thicker than for the normal, and looks much like a continuation of the ectoderm of the central flattened plate. This condition certainly suggests that this ectoderm of the germ-band was destined for the central nervous system, but owing to the lack of concentration of the nerve-plate it remained accumulated in the germ-ring.

The most remarkable modification that I have found is shown in Fig. 34. The yolk is exposed only at the circular blastopore (*y*). The blastopore is surrounded by a thick homogeneous ring. Beyond this, over the extra-embryonic region, is a thin layer of cells. The cap of cells of the early blastoderm must, therefore, have grown over the yolk equally on all sides without the differentiation of any embryonic portion. If this is true, we see why the germ-ring should be so enormously thick. Sections were made through this embryo (Fig. 43). They show that a large tongue of cells turns in around the whole inner perimeter of the thickening (*i.e.*, in the blastopore). The tongue is deep and composed of a large number of cells. It is much larger than the tongue of the germ-ring of normal embryos. The ectoderm of the ring is quite thick, and thickest at its inner edge. The ring seems to be the same throughout, and shows no bilaterality.¹

In Figs. 37 and 38 is shown an embryo in which the germ-ring on one side has failed to grow, while on the other side (right) it has retained its normal relation to the embryo. Nevertheless, the embryo seems to have elongated posteriorly and preserved its bilateral structure.² The darker portion of the figures (to the left) shows the remains of the germ-ring of that side. Sections through this embryo show that the *mesoderm* in the anterior portion of the body is equally developed on the two sides. In the middle and posterior portions there is a smaller amount of mesoderm on the left (defective) side than on the right (Fig. 42).³ This mesoderm of the left side

¹ Lereboullet has figured a similar abnormality, in which the ring has a thick process from one point. Rauber has described an embryo similar to the one figured.

² Unfortunately, the record of the previous history of this embryo was lost. Whether it was the result of some artificial solution or not I do not know.

³ The figure has right and left reversed.

is made up of fewer cells than on the right side, and the cells are individually larger on the left side, but not sufficiently so to make equal the right and left mesodermal masses. The notochord and nerve-cord are apparently normal and bilateral. The sections show, also, that the defective germ-ring is composed of loose rounded cells, with a few irregular cells beneath.

Now it is possible in this embryo that the same cause that produced the defect in the germ-ring also produced a defective mesoderm on the same side, and that there is no cause and effect between these two phenomena, but both are due to the same cause. The sections, however, taken in connection with all of the preceding evidence, give one the impression that the small amount of mesoderm of the left side is due to the absence of the germ-ring on that side.

A study of these abnormal forms leads to the same conclusion arrived at from a study of the normal development. The embryos drawn in Figs. 32 and 33 point definitely to the conclusion that the mesoderm of the germ-ring is the same in part as the mesoderm of the embryo. The dilute solution does not seem to affect this layer. On the other hand, these same embryos show that the middle axial plate of cells is destroyed or injured by the solution. When this plate is destroyed or injured, the posterior elongation of the embryo is likewise modified. Embryos like those of Figs. 35 and 36 show, moreover, that the axial plate may elongate in the posterior direction independently of the germ-ring, and extend beyond it; while Figs. 37 and 38 show very conclusively that the embryo may elongate posteriorly independently of the germ-ring of one side. The experimental evidence, as gathered from a large number of observations, of which Figs. 36 and 40 are illustrations, points to the same conclusion, *viz.*, the possibility of a nearly symmetrical axial elongation independent of the germ-ring.

HISTORICAL AND CRITICAL REVIEW.

It is not my intention to enter into a long historical account of the various theories of the method of formation of the fish-embryo. I shall attempt to touch only on those points that seem to me to be essential.

Henneguy (2) has given an excellent and brief account of the history of the subject, and Hertwig (3) has given a longer disquisition on the same topic.

Henneguy has stated very clearly the standpoint of Kupffer, Oellacher, His, Rauber, Cunningham, and Balfour, in respect to the theory of concrescence, so that we need not here dwell further on their views. There is one result, however, that must be taken into account. His has shown that the volume of the young fish after the closure of the blastopore, plus the volume of the extra-embryonic region (ectodermal wall of the yolk) and germ-ring, is about equal to the volume of fully formed blastoderm. In other words, the protoplasmic substance of the later stages is no greater than that of the early stages. The material of the germ-ring, therefore, must pass either into the extra-embryonic region or into the embryo. His' measurements go to show that the volume of the developing fish slowly increases, while that of the germ-ring decreases. It follows, he thinks, that the fish grows at the expense of the germ-ring. But it does not, I think, necessarily follow, as he believes, that the whole of the elongation of the fish embryo is directly due to addition to its posterior end from the germ-ring.

Henneguy, by a series of careful measurements of the embryo of the trout, has shown very conclusively that other factors than the germ-ring are at work during the elongation of the embryo. At an early stage, when the medullary folds are forming, and before the embryo has gotten half its full length, Henneguy measured the distance from the posterior end of the embryo to the anterior tip of the notochord. Let us call this distance *AC*. Similarly, the distance from the posterior end of the body to Kupffer's vesicle we may call *AB*. From the anterior end of the notochord to the anterior end of the head we may call *CD*.

These measurements show that the total length of the embryo from this stage till the closure of the blastopore increases by 1.90 mm. The distance separating Kupffer's vesicle from the posterior end of the tail-knob (*AB*) only increases by .055 mm. — an insignificant amount when compared with the total elongation. Therefore, Kupffer's vesicle retains its posi-

tion throughout the elongation of the embryo. The distance *CD*, also, does not increase sensibly during the period of the elongation of the embryo. The main increase is in the region *BC*, lying between Kupffer's vesicle and the anterior end of the notochord.

The number of protovertebrae increases rapidly. At the first stage about 3 or 4 pairs are present; at the last stage, about 24 pairs. Fol found in the chick, by marking the blastoderm, that the first protovertebra retains its position, and the rest form posteriorly. Henneguy's measurements show the same to be true for the fish. Since the anterior pair of protovertebrae retain a constant distance from the anterior end of the notochord; and since the size of the protovertebrae as they form remains the same; and since the distance between the last protovertebra and the end of the embryo remains *about* the same,—it follows that there is a growing region between Kupffer's vesicle and the last protovertebra formed. Here the elongation and, *pari passu* with the elongation, the cutting off of new parts of protovertebrae takes place.

Lereboullet (8) described, in 1863, certain abnormal embryos in which the right and left sides of the body were separated from one another by an exposure of yolk.¹ The anterior and posterior ends had their right and left sides united so that only the middle portion of the body had its halves separated. At a later stage the halves in the middle region united to one another. Lereboullet interpreted this to mean that each half of germ-ring developed in the normal embryo into a corresponding half of the embryo, and in three abnormal embryos the union of the halves had been retarded, and did not take place until the organ had differentiated. Each half ring, therefore, formed its half organs *in situ*.

It is clear from Lereboullet's account that he anticipated, by ten years, the conception of the concrescence of the fish embryo, usually ascribed to His. Oellacher (11), in 1873, rejected Lereboullet's theory, and believed that elongation took place "auf das raschere Längswachsthum des Medullarrohres und auf seinen Bildungsmodus. . . . das Medullarrohr

¹ The protruded yolk was really covered by a thin layer of ectoderm (!).

ist zuerst ein solider im Durchschnitte keilförmiger Strang der aus dem mittleren Theile des Sinnesblattes herauswächst. Würde dieser Theil des Sinnesblattes gespalten oder trifft der Medullarstrang im Anfange seiner Entwicklung auf ein Hinderniss, so wird auf eine Strecke ein von unten her gespaltenen Medullarstrang entstehen müssen. . . . Ich habe es hiermit zugleich auch ausgesprochen, dass nicht bloss eine Laesio continui in einem Keimblatte, sondern auch ein auf dasselbe wirkender Druck oder Widerstand eine Duplicität des aus demselben sich entwickelnden Organes bedingen kann."

If, then, elongation is due to an axial extension posteriorly, he said, and the splitting of the embryo into right and left halves is caused by division of its growing point, we have a better explanation (than on the alternative hypothesis) of the observed fact that very often there is an inequality in the amount of material in the two divided parts.

Professor Whitman's (17) masterly description, in 1878, of the process of concrescence in *Clepsine* has furnished the strongest support of the possibility of concrescence that has been published. He demonstrated the presence of concrescence for the leech, and it is not surprising that, with this definitely established case before them, embryologists have extended the same conclusions to other groups. Whitman says: "The germ-bands in *Clepsine*, their epibolic growth and final conjunction at the median neural line are so remarkably similar to the embryonic rim and the process of neuralation in vertebrates as to indicate a fundamental relationship. This similarity has already been noticed by Semper and Hatschek, and adduced as an argument in favor of a genealogical relationship between the vertebrates and invertebrates. Of the justice of the comparison I am thoroughly convinced, and I propose here to add some considerations in its favor which have, until now, passed unnoticed." Whitman's work stands as the best ascertained case of concrescence by apposition; but, from the facts stated in the preceding pages, I do not believe that we are now justified in extending exactly the same explanation to the development of the fish. Rauber (12), in 1880, commits himself to these statements in regard to the process of formation of the

Teleost embryo. The blastoderm flattens, and its perimeter thickens to form the germ-ring. From the very beginning the germ-ring is thickened at one point, and this differentiation becomes more marked while the anterior embryonic foundation is formed. The elongation of the embryo behind this point is due to the union of the two germ-bands that had before been separated. The middle portion of the embryo is added in this way to the head, and the posterior portion to the middle. "So wird aus einem Ringtheil des Keimes ein Achsentheil des Embryo. Die totale Embryonalanlage ist also das Ergebniss eines Conjunctionsphänomens." The formation of the embryo along one meridian is due to unequal growth of different portions of the embryonic material.

This interpretation of the normal development Rauber believes necessary to account for the various forms of abnormalities described in his paper. This view of the concrescence of the embryo is practically the earlier view of His.

Rauber has figured quite a number of most interesting abnormalities, and has attempted to explain, on the concrescence theory, many of the results as due to a "Conjunctionsphänomens."

There are two main classes of these abnormal embryos. In the first class we may place those embryos where, *apparently*, the two sides of the embryo are laid down separately, and at some distance from one another, united anteriorly by the anterior end of the medullary plate. An inverted V-shaped structure is produced with the two limbs of the V running out into the germ-ring. Rauber attempts to explain this condition as the result of an imperfect *apposition* of the two sides of the embryo, *due to a lack of union of the right and left sides of the germ-ring. The yolk, however, is not exposed between the two limbs of the V, as it should be if his hypothesis is correct.* But there is even a more serious objection to Rauber's interpretation.

I have tried to show that the anterior end of the fish embryo does not form by the apposition of the germ-ring, but that it is laid down before the germ-ring has begun to grow over the yolk, and that this portion of the embryo reaches from the germ-ring, where it is most thickened, to the apex of the earlier blastoderm, forming *in situ*.

It seems to me, therefore, that Rauber's explanation will not hold good, at least for the anterior end of these abnormal fish embryos. The following alternative explanation is suggested. The results may be due to a defect in the formation of the neuro-chordal plate which, instead of concentrating in the axial line, remains flattened out, or thickens at the two sides, producing, in connection with the mesoderm, the lines of the V. Such an explanation commends itself to me, because, in the first place, it will account for the presence of the layer of cells between the arms of the V; in the second place, because the sections through these embryos, which Rauber has figured, do not show a thickened ectoderm at the limbs of the V, but only a special accumulation of mesoderm; and in the third place, because such abnormalities are directly comparable to those that I have found to form when the sea-water is diluted.

I advance these views, not with the intention of setting up an alternative merely formal explanation, but with the hope that these abnormal forms may be reëxamined before they are admitted as evidence in favor of a theory of concrescence by apposition.

In the second category of abnormalities are those cases of double embryos on the same blastoderm. Rauber has shown many cases where two embryos are formed, simultaneously, on the same disc. They may appear near together, or else lie at opposite points of the germ-ring, or at any intermediate points.

Rauber believes, and I think his evidence is sufficient, that when these embryos appear near to one another, they gradually approach as they elongate, and we have formed a double-headed embryo with a single body. The proximity of the embryos at the beginning will determine the length of the free portions, since the nearer together they are, so much the sooner will they fuse.

Rauber holds this to be a demonstration that the body of the fish, as it elongates posteriorly, continually receives additions from the germ-ring to its two sides.

I see no escape from this conclusion, although, as I shall attempt to show, I do not see that it demonstrates that the posterior elongation is due to apposition.

If we do *not* assume that material from the germ-ring passes into the embryo, we should expect that two embryos, appearing near to one another on the germ-ring, would, during the early extension of the blastoderm over the yolk-sphere, separate farther and farther from one another, till the equator of the egg was reached. Rauber's evidence, which seems to me, as I say, to be valid, points clearly to the tendency of the embryos to grow together, as they grow older.

We might assume an innate attractive force that tended to draw such embryos together, but embryology has passed the period when such explanations are acceptable.

Therefore, accepting Rauber's facts, is there an escape from his conclusion? It seems to me there is. In the preceding pages I have attempted to show that there is evidence pointing to the conclusion that material from the germ-ring does pass — perhaps continuously — from the germ-ring into the embryo. But I have also tried to show that this material forms only a small part of the elongating embryo, and does not itself give rise, by *apposition*, to the entire right and left sides of the embryo.

Therefore, since the material of the germ-ring does pass into the embryo, we can accept a part of Rauber's conclusions and thus account for the union of two embryos starting near to one another on the germ-ring. The remainder of Rauber's conclusion, *viz.*, that the facts demonstrate elongation by apposition, or concrescence, does not, I think, follow from his observations, or fit in with what seems to be the normal method of the formation of the fish embryo.

Rückert, in 1887 (14), working on the Torpedo, makes the following statement: "Sonach kann ich die schon im Jahre 1876 von His vertretene Anschauung, nach welcher die axiale Anlage der Haie aus einer Verwachsung der sich einfaltenden Blastodermränder hervorgeht, für einen beschränkten (hinteren) Abschnitt des Embryo bestätigen. Dass die ganze Embryonalanlage, wie His dies will, auf solche Weise entsteht, glaube ich allerdings nicht annehmen zu dürfen." "Vom Standpunkt der Gasträatheorie betrachtet, stellt derselbe in der That einen hochwichtigen Akt, nämlich die Schliessung des Blastoporus dar."

Rückert conceives the whole periphery of the germ-disc to represent the blastopore, but only one portion of this to pass into the embryo. This takes place by a process of concrescence which serves to form the posterior portion of the embryo. If I mistake not, this is the same view which Hertwig, in 1892, advanced as his conception of the process of concrescence; but this paper of Rückert's is not quoted in Hertwig's extensive bibliography (3).

In my preliminary paper on the development of the fish, I described the results of the experiments of cutting the germ-ring on one side of the embryo. At this time I was not aware that the same experiment had been successfully done by Kastschenko (7) in Selachians. To him, therefore, belongs the credit of having first shown that under these conditions the embryo continues to elongate posteriorly.

Kastschenko cut the germ-ring to one side of the embryo (stage VII). Nevertheless, a normal embryo developed that lived through the next stage (VIII). In another experiment the posterior end of the blastoderm was destroyed. The anterior half of the embryo developed normally, but the posterior half did not develop. The development of the posterior half ought still to have followed, if His' hypothesis were true, because the material for the latter was left uninjured in the germ-ring.

In a third experiment of Kastschenko's the entire embryo (at stage VII) was separated at the two sides, from its connection with the rest of the disc, and both halves of the body continued to develop up to the time of appearance of three protovertebrae. In some cases the growth of the two halves of the embryo continued till the tail-folds appeared.

The results of these experiments, Kastschenko says, convinces him that His' theory is not true, and that the material for the formation of the axial portion of the embryo's body is laid down from the beginning, not in the germ-ring, but in the hinder end of the germ-disc.

It will be seen that my own experiments of a similar nature on the Teleost have led me to a like conclusion. In my earlier paper I inferred, as does Kastschenko, that the germ-

ring did not, perhaps, contribute at all (or to *any great degree*, as I then stated) to the formation of the embryo. Further work has however convinced me that, to some extent, the germ-ring does take part in the formation of the growing embryo, and such results are, I think, in full harmony with the results of both of our experiments. Since only a small part of the posterior elongation is due to material from the germ-ring, and the greater part to the elongation of the tail-knob, we should still expect to find, when the germ-ring is cut off, that sufficient material remained in the tail-knob to form nearly a perfect embryo. My own view also explains the results obtained by destroying the tail-knob. In such cases the major part of the embryo is injured, and its backward growth prevented, hence, since the germ-ring remains still attached to this fixed posterior end, we can understand why the material of the germ-ring does not continue to pass into the embryo. I had performed this same experiment in Teleosts, but with varying results. In some cases the embryo did not grow any farther. In other cases the embryo healed its injury and continued to extend posteriorly.

Roux, in 1888 (13), described the result of some experiments on frogs' eggs. Definite portions of blastulae were injured with a needle, and the results recorded. At the blastula stage injuries in the first foundation of the gastrula lip gave defects in the cross-commissures of the brain. Injury on the equator gave defects nearly in the middle of the medullary folds. Injury in the middle of the white pole produced no defect in the embryo, but if eccentric, a more or less imperfect union of the medullary folds was produced (*Asyntaxia medullaris*).

From these experiments Roux concluded: that the medullary folds form, in the frog, over the white pole: that the dorsal lip of the blastopore grows over the white pole through about 170° : and that this is brought about by the apposition of the two sides of the blastopore.¹

After a statement of His' concrescence theory Roux concludes: "Diese Angabe über die Bildung eines Knochen-

¹ Die Gastrulation des Froscheies vollzieht sich also wesentlich durch Ueberwachung des Aequators aus, also durch "bilaterale Epibole."

fischembryo steht, wie man sieht, durchaus in Uebereinstimmung mit den Folgerungen, die aus meinen Versuchen am Froschei sich ergeben haben. . . . Wir können daher den Satz aufstellen: Die schwarze am Eiäquator angelegte Urmundlippe des Froscheies entspricht dem Randwulste der Knochenfische. Das Material für die Medullarplatte des Froscheies liegt in und wohl noch neben dem ganzen, das Ei rings umziehenden Umschlagsrande des Epiblast in den Hypoblast."

Further, Roux has recorded cases of abnormal embryos, in which the medullary plate appears as a ring encircling the equator of the egg.

I have been able to produce the latter kinds of embryos artificially (10) by the addition of a definite amount of salt to the water during development. From a study of a number of such embryos I have tried to show that we cannot conclude, from what happens in such cases, what the normal processes really are. My reason for this statement was that in many such embryos the open ring may include a length far greater than the length of the normal medullary plate.

Later (9) I suggested that, in such cases, the medullary plate at the dorsal lip was prevented from growing along its meridian, and therefore, its substance had divided into two portions which migrated around the egg equator, to form the medullary plate and notochord. This is the same suggestion which Oellacher advanced to account for similar embryos of Teleosts, modified in the same way as the frog.

In regard to Roux's experiments, where definite points of the egg were injured, I admitted that, if verified, they gave strong evidence for Roux's conclusion.

During the present spring, 1894, I have made a very large number of experiments on frogs' eggs, and have reached the conclusion that Roux's position was well taken. I have found that injuries made laterally, just outside of the black-white line, do pass up into the medullary plate, and ultimately may reach nearly the middle line of the embryo.

I have, further, made many experiments by destroying the dorsal lip of the blastopore. From these we see that even

when the dorsal lip is destroyed, and its backward growth prevented, nevertheless, the medullary folds come up from the sides to form in the middle line, posterior to the point of injury, the central nervous system and notochord of the embryo. There is often left a large yolk-plug, either just behind the anterior cross-commissures, or more posteriorly, in the middle of the medullary plate.

It is possible that when the dorsal lip of the blastopore was injured in the middle line, its material has divided and extended backwards on each side along the black-white line.

In order to test this I operated on a number of frog embryos, destroying not only the dorsal lip in the middle line, but also making a series of injuries on each side of the dorsal lip as well. By this means the possibility of a division of the central mass into a right and left half would seem to have been prevented. Under these circumstances the blastopore closes laterally and behind. Serial sections were made to see if by this closure the normal structures were formed, or if the closure had nothing to do with the normal process. The sections showed beyond a doubt that the right and left sides of the blastopore had brought up to the median line the right and left sides of the embryo.

These and other experiments that need not be described here have convinced me that the material for the embryo of the frog is laid down in a ring of cells around the black-white line, and that this ring approaches the mid-line during the closure of the blastopore, *i.e.*, the material reaches the mid-line from before backwards. From the evidence gained by these experiments, we are forced to conclude that in those cases where the embryo formed as a ring around a large yolk exposure, the greater elongation of the sides of the embryo is due to the material differentiating *in situ*. The material for the nervous system and notochord must form a ring quite far up on the sides of the blastula. The ring is not, however, as a large series of new preparations show me, placed so far up as the equator of the egg.

The bifurcation in the spina bifida embryos is caused, then, as Roux and Hertwig state, by a failure of the two sides to

overgrow the yolk and to a differentiation of the retarded lips into the halves of the embryo.

If, therefore, in the frog, spina bifida is due to the imperfect concrescence in the two sides of the blastopore, are we to conclude that, when we find spina bifida in the fish, the germ-ring also forms the embryo by a process of apposition? I think not. It is useless, however, to discuss the matter without additional evidence. I cannot let the question pass, however, without this statement: The axial concentration of the material to form the embryo would supply the basis for an explanation of a doubling in the fish. Again, it is a most significant fact that while in the frog embryo with spina bifida the yolk-cells are always exposed, yet we find in the fish, in all cases figured, a bridge of ectoderm connecting the separated sides of the embryo. We must not, moreover, forget that the fish and frog are formed on different parts of the egg; and that finally, in the development of the frog we do not find any large amount of tissue laid down in the head region before the closing in of the blastopore begins. These facts go far towards freeing one from the supposed necessity of finding *exactly* the same explanation for the two cases of spina bifida.

His (6) has recently (1891) reiterated his belief in the concrescence theory. He says the *length* of the head of the embryo-fish remains the same when the embryo is 1.2 mm. and when it is 4 to 5 mm. Further, the early blocks of mesoderm retain the same relative position throughout the development. "Somit findet das Körperwachstum nicht durch eine innere Dehnung der ersten Anlage statt, sondern dadurch dass den zuerst vorhandenen Teilen von hinten her neue sich anfügen. Das Wachstum geschieht mit anderen Worten nicht durch Intussusception, sondern durch Apposition."

It is impossible to think, His adds, that a sudden increase of new material takes place during overgrowth, for he has shown (in 1878) that the amount of material at the beginning and end during the overgrowth is the same in amount, counting both germ-ring and embryo.

His speaks in this paper of a large amount of lateral compression taking place in the early embryo, since the breadth

decreases from 1.2 mm. to 0.55 mm., *i.e.*, as 100 to 40. His does not believe, apparently, that this has anything to do with the elongation of the embryo posteriorly. He extends his theory of concrescence to higher groups, and rejects the conception of a coincidence of blastopore and germ-ring in Sauropsida and mammals (see Hertwig (3)).

In the discussion that followed His' paper (6), in 1891, Rückert (15) states that he believes that material from the germ-ring passes into the embryo ("in die axiale Anlage aufgenommen werde"). He rejects the results of Kastschenko's experiment (7), because he has himself also cut the germ-ring of *Pristiurus* at one side of the embryo, and has carried such embryos to a later stage than did Kastschenko. He finds "eine geringere Ausbildung resp. einen Defect auf der operierten Seite (bei oberflächen Betrachtung gesehen)."

Sedgwick, in 1892 (16), has described the Elasmobranch development and given his idea of the meaning of concrescence. He says: "It must be clearly understood that the growth of the whole edge of the blastoderm has so far been a uniform one. The indentation in the embryonic rim advances equally with the more prominent parts of the embryonic rim called the caudal swellings. There is no reason to suppose that this advance of the indented part of the embryonic rim is due to the fusion of the divergent caudal swellings. On the contrary, there is every reason to suppose that the indented part of the embryonic rim advances by growth of its own substance, just as do the other parts of the edge of the blastoderm." "Further, it is clear from what I have said above that the notch of the embryonic rim represents the anterior end of the blastopore, and that, on the view of embryonic growth above stated, the blastopore does at one time or another perforate the whole length of the medullary plate. . . . Anteriorly it keeps closing up as the embryonic rim grows backwards, so that it is never present in this region as more than a notch." "It will be maintained by some that this view of the growth of the embryo and of the relation of the blastopore to the medullary plate is incompatible with the objection to the concrescence theory above formulated. To this the reply would

be that the body of the Elasmobranch embryo is no more formed by the fusion of two lateral halves than is the body of the Peripatus embryo, in which nearly the whole of the ventral surface is at one time traversed by the long blastopore.

"The phenomena we are in both of these cases dealing with is the closure of the blastopore ; and to talk about concrescence and fusion of two halves is merely obscuring the real question and seeking to explain a process of growth by a phrase which has no satisfactory meaning."

Sedgwick, therefore, holds that the embryo grows backward along two growing lines that are parallel, and end in the tail-knobs. Between these halves an imaginary blastopore is always closing up, so that the blastopore may be spoken of as perforating the whole length of the embryo. Truly, one might turn his own words against him and say that this is "merely obscuring the whole question." Whether we accept the theory of concrescence of the embryo or not, it seems unfair to speak of the conception as "seeking to explain a process of growth by a phrase which has no satisfactory meaning." Even if the phrase is unsatisfactory to Sedgwick, I cannot but believe that, as used by Lereboullet, His, Roux, Hertwig, and Whitman, the "phrase" has a very clear and definite meaning.

Hertwig has given a special section of his important paper on "*Urmund und Spina bifida*" to the Concrescence theory. He says : "Wenn ich daher jetzt zum ersten Mal über die Concrescenztheorie mich ausspreche, so muss ich zuerst hervorheben, dass ich im Gegensatz zu vielen andern Forschern in der Beobachtung von His, dass sich bei Fischembryonen die Keimwülste allmählich von vorn nach hinten zur Formirung der Axenorgane zusammenlegen, eine sehr wichtige Entdeckung erblicke. . . . Wenn His versucht, die Concrescenztheorie von der Urmundtheorie getrennt zu behandeln, so muss ich hierzu bemerken, dass die erstere nur in Verbindung mit der letzteren überhaupt verständlich wird."

We need not here enter into a discussion of Hertwig's general conception as to the process of concrescence in the different Vertebrate forms, but we may examine in some detail his conception of the process in the Teleost.

I may say at once that I do not believe his view of the subject to have been a happy choice, and, further, I think Hertwig has failed to give sufficient weight to some of the most important facts of teleostean development. Hertwig says: "In my conception of the gastrulation processes in the teleost egg I agree with His to this extent, that we both look upon the back of the embryo as formed by the union of lateral halves. In other respects I differ from him on very important points. According to my view, the whole appears as a modified gastrulation process. The parts that unite are the lips of the gastrula mouth, and the periphery of the germinal disc (*Keimscheibenrand*) is gastrula mouth only so far as it takes part in the fusion process [apposition]. . . According to His the structures that lie later in the median plane of the body form at first the whole periphery of the germ-disc. The whole border zone of the disc (*Randwulst der Keimscheibe*) is used up to form the embryo, and it is used up at the same rate that the overgrowth takes place, so that the embryo is completed at the same moment the yolk-sphere is covered." "Oellacher has brought forward this objection to Lereboullet's theory (which is very similar to that of His): If the rim of the germ-disc forms the two halves of the body, then must these halves run around the whole egg and at one time form a great circle of the sphere. Therefore, the two halves of the body must be at one time enormously drawn out, which is certainly not very probable." Hertwig adds: "This difficulty is absent from my interpretation, and I can justly say the concrescence theory of His appears in my '*Urmundtheorie*,' but in a form with modifications that are not unimportant."

It seems to me that Hertwig has completely misinterpreted the facts of teleostean development; that his "not unimportant" modification of His' theory lies entirely in a wrong direction. While I agree with Oellacher and Hertwig in their criticism of His' view of the germ-ring as actually representing the two halves of the embryo, I also think that Hertwig has gone much farther from the actual facts than even His has done.

The homogeneity of the germ-ring will be apparent to any one who has studied the development of a fish, and Hertwig's

artificial division of it into two parts, Urmundrand and Keimscheibenrand, one equivalent to the gastrula mouth, the other not (the process "a modified gastrulation process"), seems to be an unwarranted assumption. The differences in the germ-ring in different regions are quantitative, not qualitative. Sooner or later the whole of the material of the under layer (and probably a part of the upper layer) of the germ-ring passes into the embryo.

The supposed insuperable difficulty of accepting this fact, which is the basis of His' theory, lies in the value which His has given to the ring itself; for in it he sees the two sides of the embryo. If, on the other hand, we accept the fact that the germ-ring slowly passes into the embryo but forms only a comparatively small portion of it, then the criticisms of Oellacher and Hertwig fall to the ground. We return thus to the discovery of Lereboullet and His, that the germ-ring does go into the embryo. To this we may add the modern conception, which does not originate with Hertwig, however, that the germ-ring of the fish is the blastopore. Finally, the results of experimental studies show us that the median portion of the fish may elongate posteriorly independently of the additions from the germ-ring; and from this median portion, early laid down on the blastoderm, develops the greater part of the body of the fish.

APPENDIX.

The preceding pages were finished and sent to the editor early in the spring of 1894. During the summer of '94 I was able to continue my study of teleostean development at the Biologische Anstalt in Helgoland. I wish to express here my indebtedness to the Director and Staff of the station for many courtesies extended to me during my sojourn in Helgoland.

It is not my intention to enter here into a full account of the results of this renewed study, but there are two not unimportant additions that I can make to the preceding pages. My account of the outermost layer ("covering layer") of the extra-embryonic region, embryo, and germ-ring, is very imperfect, owing to the difficulty of recognizing this layer in the older embryos. The layer becomes exceedingly thin in the later stages, and in sections forms only a thin membrane over the other cells. I found during the summer of '94 that if the eggs of *Ctenolabrus* were killed by weak osmic acid, then thoroughly washed in distilled water, placed in a solution of 1% silver nitrate until they were made brown, washed, and opened in dilute glycerine, very beautiful surface preparations could be obtained.

Without figures a detailed account of these preparations would be unprofitable. Only the cells of the outermost layer ("covering layer") are stained by this method. It is found that these cells, individually, increase very much in surface area as the blastoderm grows over the yolk. Moreover, these cells divide during the same period, as the shape of the outlines of many of these cells shows. We often find pairs of rounded cells indicating that cell-division has just been finished at these points. In other preparations, where a different method has been used (chromic acid), the karyokinetic figures themselves are sometimes seen in the cells of this layer.

I have not yet calculated whether the increased area of each cell will, when all the cells are taken together, account for the increasingly larger area covered. This is probably true; however, because there is no evident source from which new cells could arise.

Stained preparations were made to see whether karyokinetic division takes place in the second or inner layer of the extra embryonic region, and germ-ring. *Cells in process of division are at all times to be found in this inner layer.* Especially numerous are karyokinetic figures in the tail-knob of the embryo. Many are also found in the

germ-ring, and, to a less degree, the cells of the extra-embryonic region are found also to divide.

Whether these divisions occur sufficiently often in the extra-embryonic region to account for the larger number of cells of that region in later stages must remain an open question. From the calculations given in the preceding pages we see that if each nucleus between the stages Fig. 2, *A*, and Fig. 6, *A*, had divided three (and a half) times the full number of cells would result. All things considered, I am inclined to believe that two processes take place in the inner layer of the extra-embryonic region, by means of which new cells are added to that region. First by cell-division more cells are produced, and secondly additions are also made from the germ-ring while the latter grows over the yolk-sphere.

The observations here recorded make it probable that *nearly all* of the material of the germ-ring, however, finds its way, ultimately, into the embryo. At first probably a larger amount passes in and later a smaller amount, but at neither period is there sufficient material in the ring to form the sides of the embryo.

Perhaps it may be best to restate here the factors that seem to me to be at work during the formation of the teleostean embryo: First there is an early accumulation of material that reaches from the centre of the blastoderm to the germ-ring on one side. *Part of this material has never been at the edge of the disc.* The material lying at the edge of the blastoderm continues out into the germ-ring on each side.

Secondly, in the so-called caudal swelling, at the posterior end of the embryo, there is at each stage a large amount of material. We find that much of this material is continually transferred backwards, because volumetric comparisons show that it is not all used up to form the regions of the embryo that lie at corresponding distances from the head. To this caudal swelling (or just in front of it) new material is always being added. This is the material flowing into the embryo from the germ-ring. When, finally, the blastopore closes, the caudal swelling has exhausted all of its material in the formation of the embryo, and, at the same time, the material of the germ-ring has passed in. Thirdly, in addition to the preceding factors that go to form the embryo, the measurements of the later stages seem to show that there is an actual elongation of the embryo as a whole during the later periods of overgrowth. This factor does not seem to be so important as the preceding two. It is possible that the material of the embryo only becomes more *concentrated* during the

later stages, producing the appearance of reduction of the material in cross-section, but I have seen no evidence in favor of this view. It is interesting to note that during the later embryonic periods the overgrowth of the germ-ring takes place very rapidly. Sections show that at this time there is very little material present in the germ-ring. It is at this time that we find the embryo elongating as a whole !

After the preceding account had been written there appeared a contribution by Locy, in the *Anatomischer Anzeiger* (No. 13, IX Bd., 1894), entitled "Metameric Segmentation in the Medullary Folds and Embryonic Rim" in the Selachian embryo. Locy finds that a superficial beading is present in the germ-ring on each side of the embryo, and he believes that he can trace this into a corresponding beading of the medullary folds. If these beadings are not the result of the hardening re-agents they show, as Locy points out, that material from the germ-ring passes into the embryo. Locy, however, goes further : "The fact that the primitive segments extend into the embryonic rim, and are subsequently drawn into the axial embryo, has an important bearing upon the formation of the latter. It serves to strengthen the view that the germ-ring represents, or originally did, the divided halves of the embryo, and that it is formed in part by their apposition. Thus the doctrine of concrescence receives support from my observations." It may be pointed out that Locy's conclusion does not follow from his premises. If his evidence prove good it shows that *some* material from the germ-ring goes into the embryo—nothing more. This, however, is also the conclusion that I have now reached in regard to the Teleost.

Locy says that the evidence from my experiment of cutting the germ-ring only goes to show that the embryo can form without the material from the germ-ring. He says the "experiments undoubtedly show that it is possible for the constructive material to be brought into the median plane, and for the embryo to elongate when entirely cut off from the germ-ring." I had considered this possibility before writing my preliminary account. An attempt to meet this objection will be found in the body of the present paper.

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DESCRIPTION OF PLATES.

| | | | |
|------------|-------------------------|-----------|------------|
| <i>E.</i> | Embryo. | <i>g.</i> | Germ-ring. |
| <i>Ex.</i> | Extra-embryonic region. | <i>y.</i> | Yolk. |

EXPLANATION OF PLATE XXIII.

(Ctenolabrus.)

FIGS. 1-12. Zeiss 2 D. Abbe camera.

Figs. 1-6, 9-12. From eggs fertilized June 9, at 7 P.M.

Fig. 1, *A*. Blastoderm at 9 A.M., June 10, 14 hrs. old.Fig. 1, *B*. Side view of last.Fig. 2, *A*. Blastoderm at 11 A.M., 16 hours old.Fig. 2, *B*. Side view of same.Fig. 3, *A*. Blastoderm at 1.10 P.M., 18 hrs. old.Fig. 3, *B*. Side view of same.Fig. 4, *A*. Egg and embryo (side view) at 3 P.M., 20 hrs. old.Fig. 4, *B*. Surface view of embryo of same.Fig. 5, *A*. Optical section, side view, of embryo at 5.30 P.M., 22½ hrs. old.Fig. 5, *B*. Posterior end of embryo and germ-ring of same.Fig. 5, *C*. Posterior end of embryo.Fig. 6, *A*. Optical section, side view, of embryo at 7.05 P.M., 24 hrs. old
(June 10).Fig. 6, *B*. Surface view of embryo of same in 3 pieces.Fig. 6, *C*. Germ-ring of same and posterior end of embryo.Fig. 7, *A*. Embryo killed in Perenyi, corresponding to 5, *A*, optical section,
side view.Fig. 7, *B*. Head of same, surface view.Fig. 7, *C*. Posterior end of same, surface view.Fig. 8, *A*. Embryo killed in Perenyi, corresponding to 6, *A*, optical section,
side view.Fig. 8, *B*. Head of same, surface view.Fig. 8, *C*. Posterior end of same, seen from inside.Fig. 9, *A*. Cross-section embryo, 7th section, at 2.10 P.M.Fig. 9, *B*. " same, 13th section.Fig. 9, *C*. " " 20th "Fig. 10, *A*. " embryo, 7th " at 3 P.M.Fig. 10, *B*. " same, 20th "Fig. 10, *C*. " same age, middle of embryo.Fig. 11, *A*. " embryo, 20th section, at 5.30 P.M.Fig. 11, *B*. " same, 31st section, in front of posterior end.Fig. 12, *A*. " embryo, 26th " at 7.05 P.M.Fig. 12, *B*. " same, 32d "Fig. 12, *C*. " same age, 35th section, in front of posterior end.Fig. 12, *D*. " " 13th " " " " " " "

EXPLANATION OF PLATE XXIV.

Zeiss, 2 AA, Abbe. Camera, $\times 85$.
 Zeiss, 2 D, Abbe. " $\times 370$.

FIGS. 13-24. *Ctenolabrus*.

- Fig. 13, *A*. Section through blastoderm, $\times 2 D$, at 9 A.M.
 Fig. 13, *B*. " " germ-ring of same, $\times 370$.
 Fig. 13, *C*. " " center of blastoderm, $\times 370$.
 Fig. 14, *A*. Longitudinal section through blastoderm, at 11 A.M., $\times 85$.
 Fig. 14, *B*. Cross-section same, $\times 85$.
 Fig. 14, *C*. Section through germ-ring, $\times 370$ (14, *A*).
 Fig. 14, *D*. " " side of germ-ring, $\times 370$ (14, *B*).
 Fig. 14, *E*. " " center embryonic shield, $\times 370$.
 Fig. 15, *A*. Longitudinal section through blastoderm, at 1.10 P.M., $\times 85$.
 Fig. 15, *B*. Cross-section same, $\times 85$.
 Fig. 15, *C*. Section through germ-ring, $\times 370$ (15, *A*).
 Fig. 15, *D*. " " side of germ-ring, $\times 370$ (15, *B*).
 Fig. 16, *A*. Longitudinal section through blastoderm, at 3 P.M., $\times 85$.
 Fig. 16, *B*. Section through germ-ring, $\times 370$ (16, *A*).
 Fig. 16, *C*. " " side of germ-ring, $\times 370$.
 Fig. 17, *A*. Cross-section through germ-ring and yolk-exposure, at 5.30 P.M., $\times 85$.
 Fig. 17, *B*. Section of germ-ring at posterior end, $\times 370$.
 Fig. 17, *C*. " " " " side, $\times 370$.
 Fig. 18, *A*. Longitudinal section of embryo and germ-ring, $\times 370$, at 7.05 P.M.
 Fig. 18, *B*. Cross-section of germ-ring, $\times 370$, at 7.05 P.M.
 Fig. 19, *A, B*. Surface view of cells of extra-embryonic region (*A*) and of embryonic region (*B*) (see text), at 6.05 P.M., 7½ hrs. old, $\times 370$.
 Fig. 20, *A, B*. Ditto, at 8.30 P.M., $\times 370$.
 Fig. 21, *A, B*. Ditto, at 9.30 P.M., $\times 370$.
 Fig. 22, *A, B*. Ditto, at 2.10 P.M. (another lot), $\times 370$.
 Fig. 23, *A-D*. *A, B*, ditto, at 3 P.M. *C*, surface cells at posterior end of embryo. *D*, surface-cells of germ-ring, $\times 370$.
 Fig. 24. Surface cells of extra-embryonic region, at 7.05 P.M., $\times 370$.

FIGS. 25-31. *Fundulus*.

- Fig. 25, *A-D*. *A*, 2-cell stage. Egg of *Fundulus*. Unequal division. Smaller cell removed. Larger cell divided. *B*, 2-cell stage ($= \frac{1}{2}$ 4-cell). *C*, 4-cell stage ($= \frac{1}{2}$ 8-cell). *D*, 8-cell stage ($= \frac{1}{2}$ 16-cell).
 Fig. 26. 8-cell stage ($= \frac{1}{2}$ 16-cell). Taken from an egg that divided normally into two equal blastomeres, of which one was removed.
 Fig. 27. 4-cell stage of normal egg with very unequal cleavage. Formed normal embryo.
 Fig. 28. Embryo *Fundulus*. Developed from one of the first two blastomeres.
 Fig. 29. Embryo *Fundulus*. Normal. Same stage as last.
 Fig. 30, *A-C*. *A*, normal embryo *Fundulus*, side view, optical section. *B* and *C*, embryos developed from one of first two blastomeres.
 Fig. 31, *A-C*. *A*, head of normal embryo. *B* and *C*, head of embryos developed from one of first two blastomeres.

EXPLANATION OF PLATE XXV.

FIGS. 31-38. *Ctenolabrus*.

Fig. 31. Embryo, abnormal, from sea-water diluted by fresh-water.

Fig. 32. " "

Fig. 33. " "

Fig. 34. Same. Without differentiation of embryo.

Fig. 35. Same. Tail-knob projecting.

Fig. 36. " " "

Fig. 37. Embryo with one side of germ-ring undeveloped. Posterior end.

Fig. 38. Anterior end of last.

FIGS. 39-40. *Fundulus*.

Fig. 39, *A-C*. *A*, young embryo. Germ-ring cut at right side. *B*, later stage of last. *C*, same, showing full length of embryo.

Fig. 40, *A, B*. *A*, young embryo. Germ-ring cut at left side. *B*, later stage of last.

FIGS. 41-43. *Ctenolabrus*.

Fig. 41. Cross-section through middle of Fig. 32.

Fig. 42. " " embryo of Figs. 37, 38, passing through posterior end of body (six sections in front of Kupffer's vesicle).

Fig. 43. Section through ring of Fig. 34.

ON THE STRUCTURE OF *BIMASTOS PALUSTRIS*, A NEW OLIGOCHAETE.

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THE earthworm of which this paper treats is not uncommon in the vicinity of Philadelphia, where it was first discovered in Fairmount Park, in February, 1893.

Since then it has been found in a number of places in Pennsylvania and New Jersey, within thirty miles of its original locality, and it will probably be found to extend, at least, throughout the valley of the Delaware River system.

It frequents the damp, soft soil on the banks of springs and streams, but has not been found in the bottom mud. It has been taken in common with *Allurus tetraedrus*, *Sperganophilus tamesis*, and several species of *Lumbricus* and *Allolobophora*. I have not found its cocoons, and the only fact that I have learned, relating to its breeding habits, is that individuals bearing spermatophores are found from the latter part of February until the first of December, when this is written.

The anatomy has been studied by means of dissections, supplemented with continuous series of longitudinal and transverse sections.

External appearance. — This worm may reach a length of three inches when fully extended, the number of somites varying from eighty to one hundred. The anterior somites are somewhat longer than those behind the clitellum, but the disparity is not so marked as in many other terrestrial Oligochaeta.

The prostomium is large and not distinctly dovetailed into the peristomial somite, being continued upon the latter by a pair of grooves which traverse about one-half of its length, and are not connected posteriorly by a transverse groove, as in *Allolobophora* and species of *Lumbricus*.

The anus is terminal, the anal somite being a little longer than the somites immediately preceding.

In the anterior region the cross-section is nearly circular, but behind the clitellum the body is more rectangular, in the living worm being almost square, with setae borne upon the angles. The setae are in four couples upon every somite, except the first, where they are wanting. They are *f*-shaped, and are nowhere modified, except to become less curved in the clitellum. The two setae of the dorsal couple are closer together than those of the ventral couple. They are arranged according to the formulae $1 - 1 > 2 - 3 = 4 - 4$ and $1 - 2 < 3 - 4$. Posteriorly, the distance between couples is more nearly equal. The nephridiopores open near the ventral couple of setae, and commence in somite IV. Dorsal pores are present, the first lying in furrow 5-6 and opening, internally, into the somite behind. There are, usually, several evident in the clitellum, and often the full number corresponding to the six somites of that region may be demonstrated.

In sexually mature individuals the body is much swollen in somites XIV, XV, and XVI, each ventro-lateral region bearing a somewhat nipple-shaped protuberance, whitish in color. These protuberances are due, partially, to the development of hypodermal clitellar tissue in this region, but principally to the great development of the prostates. The external orifices of these glands, the male genital-pores, are situated latero-ventrally upon the posterior portion of somite XV, close to furrow 15-16. The aperture is slit-like, and is surrounded by a lustreless area, which makes its position very conspicuous.

The regularity of the segmentation is somewhat interrupted and disarranged in this region, the intersegmental furrow 15-16 being evident only dorsally, and in the middle region ventrally; in the ventro-lateral region it is almost obliterated, but may still be demonstrated in sections. Furrow 14-15 is curved forwards, and 16-17 backwards around the base of the prostatic prominence. Upon somite XIV, at the summit of another, but much smaller papilla, is the oviducal pore, an oblique, slit-like aperture near furrow 14-15, and internal to

the male pore. Both pairs of orifices lie between the two couples of setae, but closer to the ventral couple.

The somites behind the genital protuberances narrow and upon XXIII to XXVIII, inclusive, is a well-marked clitellum, which is complete ventrally, although thinner, and usually showing the intersegmental furrows quite distinctly.

Anteriorly, *B. palustris* is of a deep pink color, owing to the vascularity of its body-walls. The color becomes lighter ventrally, whilst posteriorly the walls are thin and pale, almost transparent, the blood-vessels and intestine being readily seen through them.

The clitellum is clay-colored.

The posterior region of this worm is readily broken, and partially regenerated worms are frequently met with.

Body-walls. — The body is covered by a cuticle of the usual character, except in the regions immediately surrounding the male pores, where it is either wanting entirely, or else is extremely attenuated and much interrupted.

The hypodermis is as in *Lumbricus* and other terrestrial Oligochaeta.

The clitellum, as above stated, occupies somites XXIII to XXVIII, inclusive. It is well marked, both in color and texture, and is continuous, though but two-thirds as deep, ventrally. The hypodermal layer is developed at the expense of the muscular portion; both the circular and longitudinal musculatures are much reduced in this region, the former, in places, being almost obsolete in some specimens.

In the clitellar tissue proper, the same cellular elements are found as in the corresponding region of *Lumbricus*, the unmodified hypodermal cells, however, being less numerous. The outer third of its thickness is occupied mainly by somewhat flask or club-shaped cells, containing coarse, refractive granules, which closely fill nearly the entire cell. The lower portion of each cell is a nucleated and nucleolated protoplasmic mass prolonged into a tail-like process, the ultimate fate of which I could not determine.

The deeper portions of the clitellum consist of long-necked, club-shaped cells containing a clear or very finely granular

material. The protoplasmic basal portions of these gland cells are prolonged into fine filaments, which join one another to form the partitions between the columns of cells, and finally ramify in the circular muscle-layer. Each of these cells, at its base, contains a nucleus and nucleolus.

The deficiency in the depth of the ventral portion of the clitellum is in the deeper layers of cells; otherwise, this portion is similar to the dorsal. The cells described are similar to those figured for *Lumbricus* by Cerfontaine (6), except that in the present species the cells of the deeper layer are more robust, and the arrangement more irregular.

This is the only region in which my preparations show the blood capillaries passing directly into the hypodermal layer.

In the first two somites the longitudinal muscles are deflected from the body-wall, and are attached to the buccal chamber in such a manner as to serve as retractors of that portion of the alimentary canal.

The longitudinal layer of muscles is thicker ventrally than dorsally, and is somewhat interrupted in the line of insertion of the setae. It has the usual bipinnate arrangement, blood-vessels and nerves running in the "rachis." The circular muscle-layer is of uniform thickness on all aspects of the body. Many of the fibres in both layers are tubular, or solid with a clear axial portion, and lie imbedded in a large quantity of connective tissue.

The dorsal and ventral pairs of setae are usually connected by muscular slips passing, on each side, internal to the longitudinal muscle-layer, and just beneath the coelomic lining.

There are also oblique fibres subtending a portion of the ventro-lateral region, between somite XVI and the clitellum. When these contract they flatten the ventral surface and produce a well-marked ventro-lateral ridge, extending from the genital region to the clitellum. These oblique fibres run between the fibres of the longitudinal system, and are similar to the "arciform" muscles which Cerfontaine (6) has figured in *Lumbricus*.

None of the setae in the region of the genital apertures are modified, but in connection with certain of the follicles, or

setigerous glands, we find some remarkable structures, which resemble the glandular masses found upon the ventral faces of certain somites in species of *Lumbricus*, *Allolobophora*, and *Allurus*.

Behind each seta of the ventral pairs, on somites XIII and XVI, we find sac-like invaginations of the hypodermis, the outlets of which lie just within the mouths of the setigerous glands. The relations of these invaginated pouches to the setae remind one of the sebaceous glands of a hair follicle. The cuticle extends a short distance into the mouths of these pouches, thins out, and disappears. The ordinary hypodermal cells wall the outer two-thirds of the pouch, which about equals in depth the thickness of the circular muscle-layer. Into the deeper third open the numerous mouths of unicellular glands, flask-shaped cells with very long and slender necks.

These gland-cells lie on the inner face of the body-wall, the cell aggregate projecting into the body cavity, and being covered internally by the coelomic lining only. They are filled with a granular material, and in the head may be distinguished a nucleus and nucleolus, somewhat clearer than the surrounding protoplasm.

The long necks of these cells, which are the more granular portions, pass, parallel to one another, in a fasciculus through the longitudinal muscle-layer, and empty, as aforesaid, into the deeper portions of the invaginated sac. The latter, in my specimens, was filled with an apparently mucoid material, which may be of utility in causing adhesion of the worms during copulation.

These glands are advantageously situated to pour out their secretion along the setae. They do not occur in the somites bearing the genital openings, nor were they met with elsewhere. They must be regarded as a simple form of multicellular hypodermal gland. The long-necked flask-cells are manifestly hypodermal in origin. That they are not of coelomic origin is probable from the continuity of the coelomic membrane over their inner surface. They appear to be similar to certain cells forming a portion of the prostate.

The hypodermis is otherwise modified in the region of the

genital openings. Running from somite XIII to XVI, or XVII, we find a strip of clitellar tissue lying between the dorsal and ventral couples of setae. The coarsely granular cells of the clitellum are not represented in this region, which, moreover, is quite detached from the clitellum proper, the hypodermis of somites XVIII to XXII being unmodified. Not a little of the swelling in the genital region is due to this increased hypodermal thickness.

Alimentary canal. — The alimentary canal is quite highly specialized and differentiated into well-marked regions.

The buccal chamber lies in the first two somites. Its lining epithelium is columnar, with oval nuclei near the free end of the cells; it is connected with the body-wall of the first two or three somites by irregular, radiating muscle-bands, derived from the longitudinal muscle-layer of the body-wall. Other than this the musculature is slight, the circular layer of enteric muscles consisting of but a few scattered fibres.

Succeeding the buccal chamber is a well-marked pharynx occupying about two somites, but appearing to be much more extensive, owing to the great displacement of the septa. Owing to inequalities in the depth of the cells of the epithelial lining, the latter in its ventral and lateral regions is thrown into ridges and papillae. Upon the dorsal wall, or roof, of the pharyngeal sac, the epithelium is of quite different appearance. The cells are of uniform depth, more slender and with a more elongate nucleus; moreover, they are furnished with cilia, whilst the other cells of the pharyngeal region are non-ciliated.

The dorsal and lateral walls of the pharynx are covered in by a dense mass of muscle, blood-vessels, and glandular appearing cells. The latter are especially abundant in somites IV, V, and VI, where they are aggregated into paired masses. In somites IV and V the respective pairs are united in the middle line, but in somite VI the masses are much smaller and are confined to the sides of the oesophagus. I have been unable to find any communication between these cells and the lumen of the canal. Similar masses of tissue surround the pharynx in other genera of earthworms.

Behind the pharynx the oesophagus gradually increases in diameter, and changes from a flattened to a circular cross-section, forming a long tubular pouch extending for five or six somites. The papilliform arrangement of the epithelium, seen in the ventral portion of the pharynx, becomes more pronounced. When viewed *en face*, the flat-topped papillae are seen to be closely packed and polygonal in shape; under a low power the interior of this region of the enteric canal has a velvety appearance. As shown in Fig. 7, which is a longitudinal section, the epithelial layer alone is concerned in this condition. The increase in surface is enormous.

Following the region just described is a structure, resembling in its general characters, the calciferous glands of *Lumbricus*. Externally, this portion of the alimentary canal is of uniform diameter, and there are no pouches such as are conspicuous in the corresponding region of *Lumbricus*, and when the walls are ruptured there is no exudation of milky fluid. Longitudinal blood-vessels, seen through the outer wall, produce a striate appearance externally. Figs. 4, 5, 8, 9, and 10, show the structure of this region.

In somite XI the oesophageal epithelium becomes erected into longitudinal ridges, shown in cross-section in Fig. 8, *a*. In the lateral regions these rugae increase rapidly in height, as may be seen in Fig. 8, *b*, which is three sections behind 8, *a*. At the same time the several ridges begin to fuse, and the epithelium, which has become ciliated, encroaches upon the lumen of the canal, and a few sections further back the latter is reduced to a mere ciliated slit, with its long diameter dorso-ventral. A transverse section in this region shows slender struts radiating in all directions from the ciliated epithelium, and connected at their outer ends with the musculo-peritoneal layer. In other words the epithelial and muscular layers, instead of adjoining one another, or being separated only by a blood sinus, have become arranged into two tubes, a laterally compressed epithelial, and a nearly circular muscular one. The two tubes are connected by longitudinal laminae, which cut up the space between into wedge-shaped cavities.

Each lamina contains a great blood sinus, expanded both externally and internally, as shown in Fig. 9. These blood-vessels have walls of their own, apparently structureless membranes with very small, flat nuclei scattered sparsely on the surface.

These lamellar vessels are supplied by circular trunks in the neighborhood of the septa, as shown in Fig. 10, which is a tangential section. The circular trunks are given off from the dorsal vessel, and may, at times, break up into a network instead of remaining single; this vascular reticulum, however, appears to be always confined to the immediate neighborhood of the septum.

The spaces lying between the laminae are lined, throughout, by a granular layer containing numerous nuclei, each with a nucleolus. The nuclei are especially abundant in contiguity with the muscular and epithelial layers.

The lining epithelium of this region consists of ciliated columnar cells, the outlines of which are distinct in their free moieties only; their bases become lost in the nucleated protoplasmic mass just mentioned.

In somite XIV the epithelium loses its cilia and becomes thrown into great ridges, shown in Fig. 8, *c*, which a little further back abruptly terminate in backwardly-directed, valve-like folds (Fig. 5, *V*).

Claparede (7) has investigated the histology of the calciferous glands of *Lumbricus terrestris*, and my observations upon *Bimastos*, whilst agreeing with his in the main, differ in certain particulars. In reference to the blood supply of these glands, and of the adjoining portion of the oesophagus, he states, briefly, that longitudinal vessels run parallel to one another and to the axis of the body, just beneath the epithelium, and that they are connected, at intervals, by radiating vessels, with a vascular net-work beneath the muscular layer.

In the present species, as stated above, each longitudinal vessel, and its connecting radiating vessels, are fused into a vascular plate, extending from the epithelium to the muscular layer, and extending the whole length of the septum between the two adjacent follicles. In places, this vascular space may

become very thin, but appears to never become entirely interrupted.

Claparede states that in *Lumbricus terrestris* the follicles are lined with glandular *cells*; in *Bimastos palustris*, as I have shown (Figs. 9 and 10), this lining consists of a finely granular, evidently protoplasmic layer, *not* divided into cells, and containing numerous nuclei, many of them lying on the surface.

I have not found the rifts, or communications, between the follicles and the oesophageal lumen, which Claparede observed in *Lumbricus*.

Claparede's figures do not satisfactorily represent the histology of this organ.

Behind these oesophageal "pouches" the oesophagus rapidly dilates to form the thin-walled, highly vascular crop. This is lined by a non-ciliated nucleated epithelium of columnar cells; the outlines of the deeper portions of these cells are indistinct and free(?) nuclei are seen beneath their bases. Scattered here and there are flask-shaped cells, containing a granular secretion (Fig. 11, *g*). The epithelial cells are of irregular height, and great moniliform blood-vessels still further add to the irregularity of the inner surface.

In the region of septum 15-16 a large valvular transverse fold arises from the dorsal and lateral walls, and projects into the enteric cavity. This fold is thick and vascular, and the circular layer of muscle being strongly developed, it forms an efficient valve between crop and gizzard. In Fig. 5, which purports to be a dorso-ventral, longitudinal section, this valve is wrongly represented. The intestine was somewhat twisted here, and the section has passed through the two lateral walls, instead of the dorsal and ventral. The valvular fold does not occur on the ventral wall.

The crop occupies about one somite, XV, but a short thin walled region exists in a portion of the following somite. This is succeeded by a muscular gizzard, occupying somites XVII and XVIII, and sometimes a portion of XVI. In structure, it is similar to the organ in *Lumbricus*, Fig. 12.

The sacculated intestine immediately follows the gizzard, and in somite XXI the typhlosole first becomes apparent.

Male organs. — There are two pairs of sperm sacs, one pair lying in each of somites XI and XII. They are whitish in color, and are the most conspicuous organs when the worm is opened. They are attached to the anterior septa of the somites in which they lie, and do not enclose the testes or funnels. Owing to their large size, the septa are much displaced and the sperm sacs appear to occupy more than one somite; in longitudinal sections, however, this appearance is readily seen to be illusive. Septum 12-13 is thrust so far backwards by the posterior pair of sacs, that the cavity of XIII is almost obliterated. The interiors of the sperm sacs are much cut up and subdivided by trabeculae of muscle and connective tissue, with contained blood-vessels. As is common in terrestrial Oligochaeta, a pair of testes lies in each of somites X and XI. They are digitate in shape and are attached to the anterior septa of their somites, into which they freely depend.

The ciliated rosettes are large, of the usual form, and are attached to the anterior face of the posterior septa of the somites which contain the testes. From each of the four a ciliated duct passes back and unites in somite XIII, with its fellow of the same side, to form a common sperm duct.

The ducts usually, in Oligochaeta, unite in the somite behind the posterior funnel; the longer independent course in this worm is noteworthy, and was noticed both in dissections and in longitudinal and transverse sections. The ducts are not convoluted after perforating the septa, to which their respective funnels are attached, and unless gorged with spermatozoa, are not obvious when the worm is opened, being imbedded in the tissues of the body-wall, particularly posteriorly to their place of union.

In somite XV the sperm-duct ascends the anterior face of the large prostate, and being imbedded in the tissue of that organ it is rarely that this portion of its course can be demonstrated by dissection.

Upon reaching the summit of the prostate it plunges into its walls, loses its cilia, its epithelial cells gradually elongate and merge into the columnar cells lining the cavity of the gland, and its lumen becomes continuous with the latter.

The prostate is very well developed and to its large size is due, in a great measure, the conspicuous swelling noticeable in the region of the genital pores. It occupies somites XV and XVI, being strongly constricted into two lobes by the intervening septum. The portion lying in somite XV is by far the larger, and almost fills that somite on each side of the alimentary canal.

The lobe in somite XVI is shorter and less robust, being confined to the anterior portion of the somite.

The lumen of the prostate lies entirely within the lobe occupying somite XV; dorsally, where it receives the sperm-duct, it is quite narrow, but in its ventral two-thirds it rapidly enlarges and extends laterally to the external pore already described. In section the cavity is somewhat triangular in shape, as may be seen in Figs. 13 and 14. Fig. 13 is a longitudinal section near the entrance of the sperm-duct, and Fig. 14 is taken from a section, external to this, through the male external pore. It is lined by non-ciliated columnar cells, interspersed with the necks of other cells which may be distinctly recognized as glandular. In some regions the columnar cells form a true lining epithelium, but in most places they are numerically of secondary importance. Where they are most abundant their outlines are quite distinct, and each is seen to contain an oval nucleus and a nucleolus.

Externally to the columnar cells is a thick wall of club-shaped unicellular glands, differing in appearance in different regions. The prostate is well supplied with blood-vessels; the muscular portion is poorly developed, and instead of forming a distinct layer, as in *Moniligaster*, the fibres are irregularly distributed amongst the gland cells.

Upon its coelomic surface it is covered by a delicate coelomic membrane, continuous with that lining the coelom generally, and dipping down between the imperfect lobules into which the gland-cells tend to arrange themselves.

In the upper two-thirds of the prostate, these glandular cells have clear, almost transparent, oval heads with central nucleus and nucleolus, and long duct-like necks which run in a mass towards the central lumen. The swollen nucleated portions

are arranged at different depths, and the duct-like prolongations are consequently of various lengths. I was unable, except in one or two doubtful cases, to follow the individual cells to their openings into the atrial cavity, but many could be traced for about half of the distance, when they became lost in the multitude; others could be followed from their orifices outwards to about the same region, and as there was no difference in appearance it is but reasonable to assume that these glandular cells open by their long necks into the cavity of the prostate. I have in many cases all but absolutely demonstrated the continuity.

Chitinous spicules, or fibres, project from the mouths of these cells into the cavity of the prostate, or as is more accurate, into the atrial cavity, Benham having shown that this cavity is atrial whilst the term prostate should, in strictness, be applied to the glandular cells only. The chitinous filaments may, in many cases, be followed some distance into the necks of the cells, which latter I shall designate as chitinogenous glands. They are the most abundant cells of the prostate, and in the region in which they open the columnar atrial cells are comparatively few in number. Fig. 18 shows a portion of this region with the chitin spicules projecting from the mouths of the cells. Fig. 15 shows a section through the prostate from central cavity to circumference.

The second form of gland cells, although constituting a large portion of the walls of the organ which I have here called prostate, are perhaps not to be accounted homologous with the prostates of other worms.

These cells are also club-shaped, and their swollen inner ends are somewhat larger than the corresponding portion of the chitinogenous cells, and also appear to be rather more distorted through mutual pressure. They are arranged in bunches, or lobes, between which the coelomic membrane passes for a short distance. The ducts, or necks, of these cells (Fig. 16) are longer than those of the chitinogenous cells, and are arranged in a manner more strictly parallel to one another. The entire cell is filled with a granular material which stains deeply, and the nucleus is noticed as a more homogeneous

body lying in the swollen end of the cell and containing a more deeply stained nucleolus. The mouths of these cells open around and just within the male genital pore. In this region the ordinary hypodermal cells appear to be absent, in consequence of which the cuticle appears to be lacking also. The entire surface appears to be occupied by the apertures of the unicellular glands, thus giving the lustreless appearance noticed in the description of the external characters.

These granular cells are in appearance and arrangement quite like those of the post-setal glands, and it is probable that they are morphologically equivalent.

In a portion of the lower third of the prostate forming a zone between the orifices of the granular cells and of the chitinogenous cells, respectively, there is a continuous lining of columnar cells with well defined boundaries and containing oval nuclei and nucleoli (Fig. 17). Just without this lining lies the mass of granular cells forming the thickness of the organ, but their ducts, instead of passing directly to the atrial cavity, pass ventralwards parallel to the lining epithelial layer, to open, as above stated, in the neighborhood of the external pore (Fig. 14).

The cells which I have designated chitinogenous glands are without much doubt derived from the general columnar cells lining the atrium. They are certainly not hypertrophied cells of the coelomic layer, for, as has been pointed out, this exists as a distinct though delicate membrane covering the glandular mass upon its coelomic surface.

The lobe of the "prostate" lying in somite XVI appears to contain the granular gland cells only.

The glandular mass surrounding the terminus of the sperm canal differs, then, in several respects from the similar organ which has been described in other earthworms. In the first place, two distinct forms of gland cells are found, lying in different regions and secreting diverse products.

I believe that the chitin-producing upper portion of the mass corresponds with the prostates of *Moniligaster*, *Acanthodrilus* (2 and 5), *etc.*, and that the granular cells opening in the neighborhood of the genital pore are to be considered in connection

with the post-setal glands in the neighboring somites, and are, perhaps, homologous with the capsulogenous glands of *Perichaeta* and other genera (3).

In *Bimastos* the muscular coat of the prostate is much weaker than in *Moniligaster*, and is scattered irregularly through the glandular mass, instead of forming a zone just without the columnar lining cells, as in *M. Barwelli* (2), or deeper in the walls, as in *M. indicus* (5).

The gland cells are also less distinctly arranged in bundles than in the forms mentioned, and in this respect are more like those in the glandular portion of the prostate of *Deinodrilus* and its allies. The swollen heads of the cells, however, show a tendency towards bunching.

Benham has furnished us with a reasonable explanation of the much-discussed relations existing between the parts just described and the atria and prostates of *Tubifex*. According to his view, the columnar epithelium and the scattered muscle fibres are the much-reduced remains of the atrial wall, whilst the gland cells are referrible to the cement glands.

As I shall show, the cement glands of *Tubifex* and the chitin-producing cells of *Bimastos* perform the same function in the economy of reproduction.

From the shape of the atrial cavity and the character of the cell products I was early led to credit this organ with the fabrication of the spermatophores, and it was with considerable satisfaction that I afterwards found spermatophores in the course of manufacture within the cavity. In many instances it was possible, in sections, to follow the chitin filaments from the mouths of the cells, or even from some distance within the cells, to the walls of the spermatophore. From their position in the atrium it would appear that the spermatophores are extruded "head" first.

Lankester (8) and others have long ago shown that the spermatophores, or sperm ropes, of *Tubifex* are formed by the secretion of the cement glands poured into the atrium as a matrix. It is generally claimed, as stated by Vejdovsky (11), that in the Lumbricidae, which have no atria, the spermatophores are secreted by the spermathecae, that is, by the female

apparatus *after* copulation. In *Bimastos*, and we must believe the same of *Criodrilus* (4 and 10), the secretion must precede copulation, and the latter consist of the transfer of charged spermatophores from the male organs to the body wall of a second individual, there to await final disposition. The whole question of the mechanism and methods of copulation in earth-worms is in an exceedingly hazy condition.

The spermatophores of *Bimastos* are usually borne in pairs upon the lateral regions of the body in front of the genital openings, usually on somites XII or XIII. One worm may bear as many as six. They bear some resemblance to the spermatophores of *Criodrilus*, but are somewhat more complex than is indicated by descriptions of that form. They are chitinous club-shaped bodies, perfectly transparent, except the head, which is white with contained spermatozoa. The upper portion is curved and the base expanded into a disc for attachment, as shown in the figure. The upper swollen portion contains a cavity divided by curving transverse partitions into a number of narrow chambers, or pockets, each gorged towards its blind end with spermatozoa.

The partitions do not extend quite across the cavity of the spermatophore, so that there is a channel across the mouths of the pockets by which they are in communication with the exterior at the free end of the spermatophore, which is irregular and ragged, and often shows a raveling out of the chitinous filaments of which it is composed. The spermatophores are very firmly attached to the cuticle, by what means I cannot say, unless the granular cells of the prostate, whose function is unaccounted for, may secrete a cement for this purpose. The position of their orifices would suggest some such utility, or perhaps the secretion of albumen for the cocoon.

When the spermatophore is subjected to pressure, as under a cover-glass, the spermatozoa float freely out, and therefore appear to be unfixed in a cement substance such as is found in the sperm-rope of *Tubifex*. In sections of the spermatophore this point is obscure.

Female organs. — The ovary is attached to the ventral portion of the hinder surface of septum 12-13, and lies, as usual,

entirely within somite XIII. It consists of a circular disc-shaped mass, prolonged at its free end into a short tail containing the most mature ova. The septum to which it is attached has been thrust backwards by the enormous development of the sperm sacs, until it roofs over the entire length of the ovary, and the cavity of somite XIII is very much reduced.

The oviduct opens internally upon the anterior face of septum 13-14, being there expanded into a ciliated disc closely adhering to the septum. Near its upper mediad border this disc is invaginated into somite XIV, carrying with it the muscular septum. There is thus formed a small globular sac opening into somite XIII, but apparently lying in the succeeding somite. It is lined with cubical ciliated cells continuous with the cells of the ciliated disc, and is covered by top-shaped cells derived from the coelomic membrane. Between these two layers lie muscle fibres in two layers at right angles to one another.

This sac is probably an ovisac, but is similar to none of the published descriptions of that organ in other worms, and it is, moreover, so small that it could not accommodate more than two or three ova at a time. Its interior is undivided by partitions or trabeculae, and the muscular layer is well supplied with blood vessels. The oviduct passes through septum 13-14, from the lateral ventral border of the ciliated disc, that is, from the portion farthest removed from the ovisac. It is ciliated for its entire length, and has an outer irregular, but mostly circular, layer of muscle and connective tissue fibres continuous with the same tissues in the septum and body walls. Blood capillaries lie imbedded in this musculo-connective tissue tunic or between it and the lining layer of columnar ciliated cells.

For a short distance, after passing through the septum, the walls are thrown into internal longitudinal ridges, principally due to irregularities in the length of the ciliated cells; at its distal portion, however, it is oval or circular in cross section. Some of the sections showed ova at about the middle of their journey to the exterior. Spermathecae are not present. Although the sections were carefully examined, I was unable to

find even such rudimentary organs as Beddard (1) has described in *Allurus*.

Vascular system.—The vascular system has not been investigated, except in a very general way. There are three longitudinal trunks running from end to end, or nearly so; these are dorsal, sub-intestinal, and sub-neural.

The dorsal trunk is single throughout, and in the anterior region gives off five pairs of somewhat moniliform contractile vessels in somites VII, VIII, IX, X, and XI. The first four of these always flow directly into the sub-intestinal trunk; the fifth pair sometimes first pass into the intestinal wall, from which they emerge laterally and thence flow into the sub-intestinal trunk. In the latter case the free portion of the vessel is only about one-third as long, and is much weaker than its fellows. In front of these lateral arches the dorsal vessel ramifies in the buccal and pharyngeal mass.

A pair of oesophageal vessels arise from the sub-intestinal trunk in somite X and pass laterally forward to supply the tissue surrounding the anterior portion of the alimentary canal.

The sub-neural vessel lies within the neural sheath, and gives off branches, along with the nerves, to supply the body walls.

A vessel in frequent communication with the dorsal trunk traverses the typhlasole.

Central nervous system.—The main features of the nervous system are as usual in *Oligochaeta*. The supra-oesophageal ganglia lie in the third somite; the first pair of the ventral ganglionic chain, in the fourth. Several large nerves are given off from the supra-oesophageal ganglia and the oesophageal ring, and go to supply the buccal muscles as well as the prostomium and the body walls of the first two somites. The first pair of ventral ganglia send large nerves forward to the ventral wall of the second and third somites. The ventral nerve cord contains the usual tubular fibres, and is surrounded by a muscular sheath containing a copious blood supply derived from the sub-neural trunk. Nerves are given off laterally to the body walls in each somite; they penetrate the longitudinal

muscle layer about in line with the ventral couple of setae, and then follow the course of the circular muscles, giving off branches to the hypodermis and longitudinal muscle bundles.

Nephridia. — The nephridia are paired in each somite, beginning in IV, where they are smaller than in the somites succeeding. The external opening is near the ventral couple of setae. These organs appear to present no differences from those of *Lumbricus*.

The principal facts treated in the foregoing paper are as follows :

1. Behind each seta of the ventral couples in somites XIII and XVI are sac-like invaginations of the setigerous follicles receiving the secretion from long-necked granular cells, the swollen inner ends of which lie within the body cavity.

2. Around the atrial cavity are thick walls of club-shaped unicellular glands of two kinds. The clear cells of the upper portion secrete chitin for the manufacture of the spermatophore, the latter being fabricated in the atrial cavity. The granular cells which form the basal portion of the anterior lobe and all of the posterior lobe of the prostate, open around the male genital pore, and resemble the secreting cells of the post-setal glands.

3. The coelomic end of the oviduct forms a ciliated disc which is directly invaginated into somite XIV to form the ovisac. The oviduct is covered by a tunic containing muscle fibres.

4. The sperm ducts on each side join far back in somite XIII.

5. A structure similar to the calciferous glands of *Lumbricus* is described and compared with Claparede's description of *Lumbricus terrestris*.

Following is a definition of the genus:

GENUS BIMASTOS, H. F. M. (9).

1. Prostomium continued on peristomium by grooves.
2. Clitellum on XXIII to XXVIII, complete.
3. Setae in four couples.

4. Certain somites in genital region with hypodermal glandular structures in relation with the setae.

5. Male pores paired on XV on papillae; female pores paired, XIV.

6. Sperm sacs, two pairs, in XI and XII.

7. Funnels and testes, two pairs, in X and XI; free.

8. Prostates, one pair, in XV and XVI; bilobed.

9. Spermathecae, none.

10. Nephridia paired.

11. Alimentary canal, complex. Ciliated oesophageal structure (calciferous gland), in several somites between XI and XV. Crop. Gizzard, one, in somites XVII and XVIII. Not constricted by septum. 1 species *B. palustris*, n. sp. Eastern Pennsylvania and New Jersey.

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EXPLANATION OF PLATE XXVI.

FIG. 1. Ventral view of anterior region. $\times 3$. ♂, male pore. ♀, female pore. *Cl.*, clitellum. 1 *a*, posterior five somites.

FIG. 2. Side view of anterior region. $\times 3$. Letters as in Fig. 1.

FIG. 3. Dorsal view of same. Letters as before. *D.*, dorsal pores.

FIG. 4. Worm opened in dorsal median line. $\times 8$. *B.*, buccal chamber. *S.G.*, supra-oesophageal ganglia. *Gl.*, glandular masses attached to oesophagus and pharynx. *C.v.*, contractile lateral trunks. *Coe.*, ciliated region of oesophagus (calciferous? gland). *Cr.*, crop. *G.*, gizzard. *I.*, intestine. *Cl.*, clitellum. *D.*, dorsal trunk. *P.*, prostate. *S.d.*, sperm duct. *F.*, ciliated rosettes. *T.*, testes. The sperm sacs have been removed to show the structures beneath.

FIG. 5. Longitudinal median section. $\times 15$. *B.m.*, radiating buccal muscles (retractors). *S.c.*, supra-oesophageal commissure. *Ph.*, pharyngeal sac. *Gl.*, glandular masses. *S.*, sperm sacs; the two pairs overlap, and consequently both are cut in each somite. *V.c.*, ventral nerve cord. *Oe.*, papilliform portion of oesophagus. *Coe.*, ciliated region of oesophagus (calciferous gland?). *V.*, valvular folds terminating this portion of oesophagus. *Cr.*, crop. *Va.*, valvular fold depending from dorsal and lateral walls. (The intestine in section shown was somewhat twisted, and was cut in the lateral portion of the fold below. There is no fold in the ventral region.) *G.*, gizzard.

FIG. 6. Longitudinal section through setigerous gland and post-setal gland of somite XIII. $\times 250$. *Coe.*, coelomic membrane. *G.*, gland cells. *S.m.*, muscles of seta. *L.m.*, longitudinal muscle layer. *C.m.*, circular muscle layer. *G.n.*, necks of gland cells. *C.*, cuticle. *S.*, seta. *S.g.*, invagination of hypodermis to form setigerous glands. *M.*, mass of secreted material in cavity of post-setal gland.

FIG. 7. Longitudinal section of anterior region of oesophagus. $\times 400$. *L.m.*, longitudinal muscle layer. *C.m.*, circular ditto. *E.*, papillae of columnar epithelium.

FIG. 8. 8 *a*, transverse section just in front of ciliated region of oesophagus. 8 *b*, three sections back of *a*. Ciliation commencing and lacunae, *Ca.*, appearing in walls. 8 *c*, a transverse section about six sections posterior to 8 *b*. 8 *d*, a transverse section in the hinder region of ciliated tract. *P.m.*, musculo-peritoneal layer. *E.*, ciliated epithelial layer. *Ca.*, lacunae between vascular lamellae. 8 *e*, section through region of valvular folds (*V.* in Fig. 5) terminating the ciliated tract. All $\times 50$.

FIG. 9. Cross section of portion of ciliated tract (calciferous glands?) to show histology. $\times 400$. *C.m.*, circular muscle layer. *L.m.*, longitudinal ditto. *B.v.*, radiating longitudinal blood vessels. *N.*, nuclei lying in protoplasmic mass on walls of blood canals. *C.*, masses of these nuclei contiguous with epithelial and with muscular layers. *E.*, ciliated lining epithelium of oesophageal tract.

FIG. 10. Tangential section through one of the circular trunks of ciliated region of oesophagus. $\times 400$. *C.v.*, circular trunk. *B.v.*, longitudinal vessels given off from *C.v.* *N.*, nucleated protoplasmic mass on walls of blood vessels. A section through this region in a plane parallel to that of Fig. 9 would show the radiating vessels connected by a great annular trunk at their outer ends.



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EXPLANATION OF PLATE XXVII.

FIG. 11. Longitudinal section through valvular fold *Va.* in Fig. 4. $\times 400$. *B.v.*, blood vessels. *E.*, columnar lining cells of crop. *G.*, gland cells. *N.*, free (?) nuclei lying at bases of epithelial cells. *M.*, muscle fibres derived from circular layer of alimentary canal.

FIG. 12. Longitudinal section of gizzard. $\times 400$. *C.*, thickened cuticular lining. *E.*, epithelial layer secreting *C.* *B.v.*, longitudinal blood vessel. *C.v.*, circular vessels between the bundles of the greatly hypertrophied circular muscle layer *C.m.* *L.m.*, longitudinal layer of muscle.

FIG. 13. Longitudinal vertical section of prostate. $\times 70$. 14-16 and 15-16 septa. *P.*, layer of chitin-producing cells surrounding atrial cavity *A.* *L.m.*, longitudinal and *C.m.*, circular, muscle layers of body wall. *Hy.*, hypodermal layer. A few sections external to this, the upper end of atrial chamber receives the sperm duct.

FIG. 14. Longitudinal vertical section of prostate, more lateral than Fig. 13, and passing through external male pore. $\times 70$. *A.*, atrial cavity. δ , male pore (atriapore). *P.*, layer of chitin-producing cells. *G.*, layer of granular gland cells. The approximate distribution and the region in which these cells open is shown by the cross-hatched area. *S.d.*, sperm duct passing up the anterior wall of prostate. 14-15, 15-16, 16-17 septa, *L.m.*, longitudinal, and *C.m.*, circular muscle layers of body wall. *Hy.*, hypodermis.

FIG. 15. Section of prostate in the region *P.* of Fig. 13. $\times 210$. *Coe.*, coelomic membrane. *P.*, chitinous glands. *N.*, necks of same, with chitinous spicules, *Ch.*, protruding from their mouths into atrial cavity. *Mm.*, muscle fibres. *E.*, layer in which lie scattered columnar cells with oval nuclei.

FIG. 16. Section of prostate through one of the regions *G.* in Fig. 14. $\times 210$. *Coe.*, coelomic membrane. *G.*, granular gland cells prolonged into slender necks, *N.*, parallel to one another.

FIG. 17. Columnar cells lining atrial cavity in the narrow zone between the orifices of cells of regions *P.* and *G.*, Fig. 14. $\times 750$.

FIG. 18. Enlarged section of region *E.* in Fig. 15. $\times 750$. *Ch.*, chitinous filaments, which in some cases may be traced into the necks, *N.*, of the chitinous gland cells. *E.*, nuclei of the columnar cells lying amongst *N.*

FIG. 19. Entire ovary. $\times 125$.

FIG. 20. Longitudinal section of ovary in outline. $\times 125$.

FIG. 21. Two well-developed eggs in ovary. $\times 325$. *F.*, follicle. *F.c.*, Aggregation of follicular cells. *V.*, vitelline membrane, which is the only covering of the eggs when discharged into the body cavity.

FIG. 22. Transverse section of oviduct. $\times 350$. *M.p.*, musculo-connective tissue tunic covering the oviduct; continuous with *S.* of Fig. 23. *E.*, ciliated columnar cells continuous with *E.* of Fig. 23.

FIG. 23. Ciliated disc (funnel) of oviduct. $\times 170$. The Roman numerals indicate the somites. *E.*, ciliated cells becoming lower in ovisac (?). *O.*, peritoneal top-shaped cells covering *O.* in somite XIV. *S.*, septum. *L.m.*, longitudinal muscle layer of body wall.

FIG. 24. Spermatophore. $\times 70$. *B.*, flattened and expanded base for attachment. *P.*, pockets separated by curved partitions and all opening into chamber *C.*, which is in communication with exterior at free end of spermatophore.

THE ANATOMY OF BDELLODRILUS ILLUMINATUS, AN AMERICAN DISCODRILID.

J. PERCY MOORE.

Introduction. — The present paper is devoted to a description of the general anatomy of *Bdellodrilus illuminatus*, without much attention to histological detail, or any discussion of the special evidence which this species affords, *pro* and *con*, on the matter of current views regarding the zoological relationship of the family. The latter is reserved for a future paper, which awaits the completion of embryological studies now in progress, as well as a more thorough knowledge on the part of the writer, of the extensive literature of oligochaete and hirudinian anatomy.

Bdellodrilus illuminatus was originally described, under the tentative name of *Branchiobdella illuminata*, in the Proceedings of the Philadelphia Academy of Natural Sciences, 1893, p. 421; since which time more thorough anatomical studies, and the examination of additional native species of Discodrilidae, and of the European *Branchiobdella parasita* (specimens of which were kindly obtained for me by my brother, Mr. H. F. Moore, from Dr. Anton Collin), have forced me to the opinion then entertained, that this species should constitute the type of a distinct genus.

This it is proposed to name *Bdellodrilus*, as being at once suggestive of probable affinities with the Oligochaeta, and of special structural modifications in the direction of the leeches.

The genus is defined by the possession of the following characters, all of which distinguish it from *Branchiobdella*. There are two pairs of testes and two pairs of vasa deferentia occupying the fifth and sixth post-cephalic somites. The anterior pair of nephridia open to the exterior by a common pulsatile vesicle. The dorsal and ventral jaws are quite dissimilar. *Branchiobdella* possesses but one pair of testes and ducts, separate nephridial pores, and similar dorsal and ventral jaws.

Of described species two belong to *Bdellodrilus*, viz.: *B. illuminatus*, and *B. philadelphicus* = *Astacobdella philadelphica* Leidy (Pro. A. N. S. Phila., 1851, p. 209). Briefly these are distinguished as follows: *B. illuminatus* — Head narrower than the tapering anterior body segments. Dorsal jaw a compressed dentigerous ridge, passing between the large paired teeth of the lower jaw. There are nine pairs of conspicuous lateral glands in the nine anterior body somites. Spermatheca slender, and bifid dorsally. Penis freely eversible. *B. philadelphicus* — Head much broader than the anterior body segments. Both jaws triangular, the dorsal provided with a single apical tooth, the ventral with a pair of smaller ones. No lateral glands. Spermatheca broad, thin-walled, and sub-cylindrical. Penis proper not eversible, but carried to the exterior by the eversible bursa, into which its projecting end is received. A conspicuous prostate, in addition to a large glandular sperm-sac.

In the matter of technical manipulation *Bdellodrilus illuminatus* offers somewhat greater difficulties than other Discodrilidae. These arise chiefly from the presence of the relatively enormous lateral mucous glands, which envelop the resting body in a mass of glairy secretion, interfering with the ready penetration of fixing fluids. The best preparations for anatomical study were obtained by suddenly pouring over active individuals hot corrosive sublimate solutions, especially that recommended by Lang for fixing planarians. The latter caused instant death and complete extension. Flemming's chromo-aceto-osmic acid mixture gives the usual excellent results as regards fidelity of fixation of histological structures, and is particularly useful in the study of spermatogenesis, for which the Discodrilidae are favorable subjects (see Voigt). For the special study of the chlorogogue cells osmic acid is useful. Perenyi's fluid, Merkel's fluid, potassium bichromate, chromic acid, and picro-sulphuric acid did not give useful results with this species, though not objectionable with *B. philadelphicus* and other species. Narcotization with chloroform was successful; while alcohol was worthless for this purpose. As to stains, either Delafield's or Kleinenberg's

haematoxylin gave the best general results. Biondi-Ehrlich was particularly valuable for differentiating glands, for tracing nerves among the muscles of the body-walls, and in studying the testes and ovaries. Borax carmine occupied its usual important place among general stains. Methylene blue was also used as a nerve tracer. The results obtained from a study of many series of sections were further verified on living material, mounted preparations of entire worms, and teased preparations.

External form. — The rounded club-shaped body is terminated anteriorly by the so-called head, and posteriorly by the sucker. The head is narrow and is less sharply distinguished externally from the following segments, than is usual in the family (Fig. 1). It consists of four annuli, which, there is reason to believe, represent as many somites. The first, or peristomial annulus is divided into very mobile dorsal and ventral lobes or lips, which exhibit slight median emarginations, but are otherwise entire; and lack the sensory hairs and papillae which are usual in this family. There is no prostomium. The fourth cephalic annulus is very narrow; and when the animal is contracted, is completely hidden beneath the overlapping anterior margin of the first body somite, into which it is drawn by the longitudinal muscles. The remaining two, the second and third, appear externally as simple muscular rings.

The body proper consists of eight strongly bi-annulate somites, posterior to which three crowded, but still obscurely bi-annulate somites, form the posterior sucker and its support (Fig. 1, 1 to 11). These increase in diameter, gradually, from the first to the seventh, and then decrease rapidly to the posterior sucker, which is part of the eleventh somite. Each of the body somites, with the exception of the last, is conspicuously divided into an anterior major, and a posterior minor annulus; the former are much longer, as well as broader, than the latter, and appear more prominently so the greater the degree of contraction, which results from the peculiar manner in which the minor annuli are telescoped within the anterior margins of the major annuli following, as well as to the more

prominent bellying of the free hypodermis when the longitudinal muscles shorten.

Of the body somites, the fifth, sixth, and seventh are the sexual; the first four and the eighth nephridial. The external opening of the anterior pair of nephridia is readily seen on the mid-dorsal line of the major annulus of the third post-cephalic somite (Fig. 1, *nv*), and the paired pores of the posterior pair in a dorso-lateral position between the eight and ninth somites. Of the three enlarged sexual somites the sixth and seventh are rendered opaque by the great development of aggregations of unicellular glands constituting a clitellum. On the fifth a slight swelling on the ventral surface of the major annulus indicates the position of the external spermathecal opening, and a more prominent enlargement of the corresponding region of the succeeding segment the mouth of the copulatory bursa (muscular atrium) (Fig. 1, *z*). The external oviholes are relatively minute apertures ventrally located on each side of the seventh somite (Fig. 1, *o*), between the major and minor annuli. The anus opens on a small lobate papilla on the dorsum of the tenth somite (Figs. 1 and 5, *a*).

Bundles of ductules arising from the huge mucous glands which occupy the lateral regions of the nine anterior segments, pierce the hypodermis, and open on the surface of the body in the dorsal and ventral quadrants of the major annuli. The sucker-bearing somites are much crowded; and bi-annulation is only obscurely indicated. The acetabulum itself is a complex muscular ring or disk, having a slight posterior concavity. Its centre lies in the same axis as the body somites, so that in a position of rest the body of this species is strongly arched, instead of resting extended along the surface to which it is attached, as is the case with the species having depressed forms and ventrally directed suckers, which live on the broad surfaces of the chelae and carapace of crayfish.

Body walls.—The body walls are made up of the usual succession of an epidermis with its continuous cuticular covering, an external layer of circular muscle fibres, and an internal more powerful layer of longitudinal muscle fibres. The delicate cuticle is quite continuous, and follows the epidermal cells by which

it is secreted into all the external folds, and the oral, anal, and sexual invaginations. It is transparent, colorless, non-iridescent, and under low powers apparently homogeneous; and maintains on the exterior a uniform thickness of .0015 mm. By prolonged maceration in water it may be easily separated as a continuous piece much larger than the body which it enveloped, and with the tubular invaginations all everted. Surface views of such preparations show very distinctly the minute perforations and the cruciform markings which so generally characterize the cuticle of *Oligochaeta*; and which Voigt has described for *Branchiobdella*. Into the oral invagination and through the pharynx the cuticle extends with undiminished thickness, to terminate a short distance within the first post-cephalic somite, after thinning to an excessively delicate ragged edge. Conspicuous dorsal and ventral hollow thickenings in the anterior region of the pharynx form the jaws. The dorsal jaw is a medium longitudinal ridge bearing on the free compressed margin three posteriorly directed teeth. This is borne by a cuticular plate which anteriorly passes into a pair of slightly divergent ridges, and posteriorly forms a single median piece, passing on all sides into the unthickened cuticle. The entire structure is molded on an epithelial thickening, over which the supporting plate is bent at an obtuse angle, the limbs of which embrace the muscular supporting pad anteriorly and posteriorly, and afford points of attachment for the protractor and retractor muscles (Fig. 9, *dj*). The maximum elevation of the dentigerous ridge is .01 mm., which is about twice the actual thickness of the cuticle at this point. The ventral jaw consists of a pair of similar parallel ridges, united posteriorly by a heavy cross bar, and passes anteriorly, after diminishing in thickness, into the unmodified cuticle. Each ridge bears at its most prominent point a strong claw-like tooth. The muscular pad supporting the ventral jaw is embraced in a manner similar to the dorsal one (Fig. 9, *vj*). The mechanism of the jaws is explained below. There is a very short anal extension of the cuticle, which reaches into the intestine scarcely to the anterior border of the anal segment. No cuticular lining is apparent in the ovipore, and only a very

delicate one in the spermatheca. The everted atrial cuticle consists of a bulbous enlargement which belongs to the bursa, and a slender tubular portion which lines the penis.

The epidermis consists of ordinary epithelial cells, of non-nucleate protoplasm, and of gland cells, which are present in great number and variety. The epidermis proper presents little modification. The cellular elements are arranged with relation to the circular muscle fibres, which encircle the body walls at regular intervals and are so deeply imbedded in the epidermis that they are frequently almost in contact with the cuticle, only a thin layer of protoplasm (less than the diameter of a nucleus in thickness) separating them. Between these muscle fibres the cells are arranged in zones, which are still further broken up into groups by communicating branches which pass between adjacent fibres (Fig. 3). The epidermis may be described as made up of small irregular groups of cells united by an external sheet of non-nucleated protoplasm; the cells lie between the muscle fibres, the non-nucleated protoplasm extends over them (Fig. 2). The entire external stratum of the epidermis exhibits a fine vertical striation, doubtless connected with cuticle secretion. (Fig. 2, *sl*.) Small spaces (*s*), extending from intercellular spaces of the cellular zones, are occasionally seen in the non-cellular regions; and dark lines, *d*, which I am unable to account for, are more frequent. The cells are cubical and possess cell walls which are well marked internally, but obscure peripherally in the striated layer. The nuclei are large and deeply staining; and possess one or usually several nucleoli. Among the cells the transparent ductules of unicellular glands wind their irregular or spiral courses to the surface. In thickness the epidermis varies from an average of .009 mm. in the non-cellular region to .018 mm. in the cellular.

Anterior to the jaws there is no modification of the oral epithelium, but within the pharynx it undergoes a marked change, owing to the encroachment of the radial muscles, which penetrate between the cells almost, if not quite (in part) to the cuticle (Figs. 9 and 11, *ep* and *rm*). Between the branching inner ends of these muscle cells the pharyngeal

epithelium extends as deeply staining nucleated processes of protoplasm, which often lose their distinct cellular boundaries. The epithelium is largely displaced ; and sections often show a predominance of muscular over epithelial elements in this layer.

Glands are very richly developed in connection with the epidermis of *B. illuminatus* ; and while all are constructed of similar elements, they differ much in the size and arrangement of these elements. The elements are unicellular glands somewhat of the goblet cell type. In most cases they consist of an enlarged irregularly polyhedral body containing the nucleus, and tapering at one end into a slender, more or less elongated, ductule. The only exceptions are those glands which are referred to as salivary and bursal glands.

Certain small mucous glands are very generally distributed over the skin ; especially on the head, where they are regularly arranged in several transverse rows. They may be unicellular, or consist of three or four unicellular glands, the ductules of which are twisted or spiral.

On the sixth and seventh somites such glands become greatly increased in number and size ; the body walls, particularly on the dorsal side, being little more than a thick glandular layer, which constitutes the clitellum (Fig. 4). The unicellular glands are here aggregated in sub-globular or pyriform groups of from three to twenty or more, which extend inward to a length of from .03 mm. to .065 mm. Being arranged in a single stratum each cell forms part of the surface of the gland, close to which lies the deeply staining nucleus, in a mass of almost as deeply staining granular protoplasm. The inner ends are granular but clear and often unstained, and pass into ductules, which may be bound together into a fascicle, and either open in close proximity on the surface, or separate and open singly. In either case they wind a slightly spiral course, which is best seen in living animals, particularly when stained with methylene blue. The cell bodies have an average diameter of .011 mm., the ductules of .0018 mm., and a total length of about .05 mm.

The several cells in each group appear not to function simultaneously. Some have completely broken down into secretion

while others are entirely protoplasmic. Voigt's figures of *Branchiobdella* show this well.

The clitellar glands are arranged in crowded transverse rows between the circular muscle fibres, which are often displaced and carried inward in a highly developed clitellum. On the dorsal side, particularly of the major annuli, the glands are much crowded and of elongated pyriform shape; while elsewhere they are more scattered and globular.

Two or three pairs of large glandular masses are developed in the pharyngeal region of the head. These are of irregular shape, and occupy the spaces between the radial muscles around the pharynx; the halves of each pair almost touch dorsally, and extend laterally and ventrally nearly to the nerve ganglia, while the several pairs are separated by strong muscular bands. The component cells are larger than those of the clitellar glands, and very irregular; they contain an irregular nucleus, protoplasmic strands, and granular cytoplasm. The ductules are exceedingly long and delicate, measuring in some cases .125 mm. or more in length. Those from the dorsalmost cells form an axis, about which the remaining cells are arranged (Fig. 11, *ag*). The groups of ductules (Fig. 11, *d*) from the several glands converge toward the ventral side of the lower lip, where they break up, and are distributed singly over the surface and margin of the lip.

Along the margins of both lips and within the mouth on the lower lip, small groups of slender gland cells open. During life these have a pale rose color and impart a decided tint to this region.

The functions of these two sets of head glands have not been certainly determined; but the habits of the animal suggest that they secrete a sticky fluid used either to aid the lips as adhesive organs, or to attach the cocoons, or both.

Glands similar to those described by Dorner, and more fully by Voigt, in *Branchiobdella*, are well developed in this species in relation to the posterior sucker (Fig. 5, *ag*). The glandular masses, which largely fill the tenth and eleventh and part of the ninth somites, are pyriform, or aggregations of several pyriform groups. Large, granular, lightly staining cell bodies

give rise to long slender ductules, which, first united into fascicles, break up into smaller and smaller groups, and are finally distributed singly on all parts of the surface of the acetabulum. This arrangement is beautifully shown in living specimens. The ductules are filled with rounded granules, which may be forced from their mouths in living worms by pressure. The granules will emerge in strings, absorb water, swell, and run together in a very short time, forming a homogeneous mucus.

Of all the numerous glands developed from the epidermis none are so remarkable as the great lateral mucous glands, whose clearness in living examples suggested the specific name for the species. Each of the post-cephalic somites, from the first to the ninth, inclusive, possesses a pair of these structures, which occupy, in the more anterior ones, almost the entire body-cavity of the major annuli, lateral to the heart, alimentary canal, and nerve cord (Fig. 7, *lg*). Of more or less spherical or broadly fusiform shape, they pass dorsally and ventrally into fascicles of ductules which perforate the body walls, and open on the surface between two circular muscle fibres (Figs. 1 and 7, *lg*). Occasionally, instead of a single gland, there are two pyriform ones, placed end to end and more or less united (Fig. 1, *lg8*), or the cells may have this grouping within a single gland. The external openings are seen in surface views as four series (two dorsal and two ventral) of clear sieve-like spots, in which the ends of the ductules appear as 10-15 clear polygonal areas in a stained meshwork (Figs. 6 and 7). The ductules have a terminal diameter of .002 mm.

The exceedingly large gland cells, which often have a diameter much exceeding that of the entire nerve-cord and ganglia, that is, about .05 mm., have an irregularly polyhedral shape; their broad bases being fitted together to form the surface of the gland, near which the nuclei lie; while internally they taper into the ductules which frequently traverse the entire length of the gland, and emerge with the fascicle at the opposite pole, often having a complete length of .1 mm. The cell protoplasm is faintly granular, and very transparent; and is very slightly, or not at all, affected by stains. Of all the stains which were used methyl green alone gave a decided color.

Biondi-Ehrlich readily differentiates between these and the adhesive glands of the acetabulum, which most resemble them, staining the former more blue, the latter more red. Alcoholic cochineal fails to stain the contents of these cells at all, while the adhesive glands are deeply colored. The cell-walls and nuclei, however, always stain deeply. The former are distinct, but delicate, sometimes tensely stretched, sometimes, notably in worms which have been inactive for a long time, wrinkled and flaccid. Most of the abundant glairy mucus which envelops the resting worm is secreted by these glands. In the quiescent condition of the worm the posterior sucker is firmly fixed, the head and anterior somites are coiled ventralward, about one and one-half turns, and the transparent mucous covering thrown out as a protection. As it leaves the ductules the secretion has an almost fibrous structure, which reminds one of a spider's thread as it leaves the spinnerets, but this soon disappears in the water.

The lateral glands probably represent the setigerous epithelium of the Enchytraeidae, *etc.*

Filling up the basal portion of the upper lip is a conspicuous gland, which may have a salivary function, and which differs in appearance from any of the glands yet described. It is developed from a tubular invagination of the oral epithelium, just in front of the upper jaw (Fig. 9, *sg*), and, in the adult consists of a single stratum of coarsely granular pyramidal cells, opening into a short tubular duct, which is somewhat eccentric in position, owing to the cells of the posterior wall having their growth restricted by crowding against the muscular jaw-pad. A slight median dorsal constriction divides the gland into two lobes. This gland is further remarkable from the presence of a muscular sheath, which extends as a delicate sheet from the dorsal region of the muscular jaw-pad, over its dorsal and anterior faces, to the oral epithelium. The sheath slips freely over the glandular epithelium and serves to elevate the lip; and, perhaps, also to aid the flow of secretion from the duct by pressure on its walls.

Glandular structures, developed especially by the epithelia of particular organs, will be described in their proper connection.

Muscular system.—The muscles of the body walls are disposed in two layers, the outer of circular, the inner of longitudinal fibres (Figs. 7 and 11). Both consist of elements of somewhat complex structure, which has been described by Voigt for *Branchiodella*; those of this species differ but slightly from Voigt's description, though they are smaller than in any *Discodrilid* which I have examined, the circular fibres having a diameter of about .0075 mm., and the longitudinal of .0075–.015 mm.

Only a general account of the muscles can be presented here, as a complete description of their distribution and variations in the different regions of the body would require the space of a separate memoir.

The circular muscle fibres do not form a continuous layer in the walls of the body somites, but are scattered singly at regular intervals, encircling the body like hoops, of which there are from 12 to 16 per somite. As before described, they are closely associated with the epidermis, in which they are deeply imbedded; and thus divide it into alternating cellular and non-cellular zones. This relation is so intimate that it is more convenient, if less accurate, to speak of a musculo-epithelial layer, rather than of a muscular and an epidermal. This is the more so because of the wide inter-muscular spaces which separate the circular from the longitudinal muscle layers; and permit to each greater freedom of adjustment. These spaces exist principally in the major annuli of all the complete somites, and extend completely around the body, except where interrupted by the spermatheca and atrium, in the fifth and sixth somites, respectively. In contraction the skin (epidermis and circular muscles) rises freely all around in a prominent fold, like an arch, as seen in optical or actual section, of which the longitudinal muscles form the cord. In extension, the two layers approach and touch, obliterating for the time the space between. The spaces are in part occupied by the smaller skin and clitellar glands, by the terminal trunks of the nephridia, the nephridial vesicle, and portions of the ductules of the great lateral mucous glands, and are further, more or less, filled by a loose reticulum of connective tissue fibres and cells. The

circular fibres (Figs. 2 and 4) consist of a more or less complete tube of a dense fibrillated (having longitudinal striae) protoplasm which bears on the internal side a bulging mass of granular protoplasm, in which the nucleus is imbedded; similar granular protoplasm fills the lumen of the cortical tube. Minute branches of the cortical substance communicate with neighboring fibres, and thus form an irregular muscular reticulum, in the meshes of which the groups of epidermal cells are included (Fig. 3). This is well shown in surface views of the skin stained in methylene blue.

The more powerful longitudinal muscle coat is continuous throughout the length of the body, and becomes, through branching and complex interlacing, much differentiated at both the anterior and posterior ends. In general, the fibres are arranged side by side in a single layer, or incompletely in two layers; in the latter case those of one layer show a tendency to slip between those of the other. Most of the fibres have a length equal to a complete somite; but they are so arranged that the ends of adjacent fibres do not coincide by a distance equal to the length of a minor annulus; that is, in any somite, alternate fibres, all around the body, extend for the entire length of that somite through both annuli, while the remaining fibres extend from the posterior limit of the major annulus to the anterior limit of the minor annulus next anterior, thus breaking joints and materially increasing the strength and flexibility of the body walls. This arrangement is particularly striking in *B. philadelphicus*, in which the muscle fibres are much larger. Where fibres join end to end there is an irregular jagged interlocking, which looks like the line of a break across the grain of a board, and doubtless is to be explained on the same principles. The fibres have a structure similar to that of the circular muscles, but are exceedingly variable in the relative amount and arrangement of striated and granular substance. Some exhibit a complete cortical layer with radiating longitudinal markings, within which, as a medullary portion, the granular protoplasm is confined with the nucleus; others show a large mass of granular protoplasm, with the striated substance variously arranged,

and perhaps limited to two small bands. Muscle fibres of the former type, but with the addition of an external granular mass, in which the nucleus is imbedded, about the middle of their lengths, are most frequently met with. Figs. 7, 8, and 11 show a few examples.

The inter-segmental septa are composed of more or less parallel dorso-ventral muscles, which form thin sheets, arising by one or two roots, from the ends of the longitudinal fibres. Where best developed the component fibres are bound together by cross-slips, much as are the circular body muscles. Septa are aborted between the first four somites, which freely communicate; but remnants remain as a few delicate slips which guy the alimentary tract to the body walls. They are well developed between the sexual somites.

The musculature of the posterior sucker is complex, and well adapted to secure strength and mobility. The circular muscles undergo little change; but the longitudinal split up, by the branching of individual fibres, into a set which are the direct continuation of the body longitudinal fibres, a second set which pass dorso-ventrally across the body cavity, a third which radiate to the margins of the disc, and, lastly, a highly branched set which have become slightly displaced at their posterior ends, right or left from their original longitudinal direction, and consequently pass with a slight spiral turn from the body walls to the periphery of the sucker, where they cross and interlace with their fellows having an opposite displacement.

Alimentary canal.—The digestive tract is distinguishable into oral, pharyngeal, œsophageal, intestinal, and anal regions.

The mouth has an extreme anterior position between the very mobile lips into which the peristomial annulus is divided. The dorsal lip is slightly the longer, and, projecting somewhat, gives to the mouth a slightly ventral aspect (Fig. 9). Labial papillae, so well developed in *B. philadelphicus* and in *Branchiodella*, are absent, a few faint ridges indicating a slight tendency toward their development. Sensory hairs are also absent. Circular muscle fibres continue unchanged into the lips to the oral invagination; while the longitudinal muscles partly continue, but mainly break up into delicate dorso-ventral and radiat-

ing fibres, which traverse the internal cavity of the lips and constitute the chief musculature of these versatile structures. The lips are bounded posteriorly by the muscular jaw pads (Fig. 9, *dm*, *vm*).

Numerous single and small aggregations of unicellular glands are developed from the epidermis of the margins and internal surfaces of the lips. Such are especially numerous on the inner surface of the ventral lip just anterior to the jaw (Fig. 9, *og*). In a similar position on the dorsal lip is the supposed salivary gland, *sg*.

Immediately posterior to these structures are slit-like dorsal and ventral infoldings of the epidermis and cuticle, bounded behind by a thickened epidermal pad. On the posterior walls of these invaginated pockets, and on the epidermal prominences which follow, the jaws are molded. The cuticle of the invagination gives attachment to the protractor muscles of the jaws (Fig. 9, *dpm* and *vpm*), which are dorso-ventral fibres derived from the anterior face of the muscular jaw pads, in addition to which the ventral jaw possesses, as an accessory protractor, a pair of muscle fibres which arise from the apex of the muscular ridge into which the jaw pad rises, and, curving around an isolated fibre posterior to the mass of the pad, insert into the posterior limb of the jaw, on which they exert a dorsal traction, and consequently move the point of the tooth forward (Fig. 9). The retractor muscles are, particularly in the case of the dorsal jaw, an extremely large and powerful pair of fibres, which spring from the longitudinal muscular coat in the region of the first cephalic constriction, and pass obliquely forward to insert, in the case of the upper jaw, directly on the posterior limb of the jaw plate, and of the lower, into the epidermis and cuticle immediately behind the jaw (Fig. 9, *drm* [the anterior index line] and *vrn*).

The pad on which the jaws are supported is a powerful muscular apparatus formed of two (a dorsal and a ventral) half disks, each of which consists of four or five semi-circular or semi-lunar muscle fibres piled upon and embracing one another, and meeting those of the opposing half disk in a transverse line (Figs. 9 and 10, *dm* and *vm*). This is set transversely in the

head at the beginning of the pharynx, and extends from the walls of the latter on all sides nearly to the external muscle layers of the head, to which it is bound by numerous radiating fibres (Fig. 10). A few dorso-ventral fibres cover the anterior and posterior faces of both plates. The circular fibres taper more or less from a nucleated middle toward both ends.

The mechanism of the jaws is seen to be powerful and efficient. The muscular plates, with their radiating fibres, regulate the distance between the two jaws, approximating or separating them as the circular or radial fibres contract in turn. The circular muscles seem sufficiently powerful to bring the jaws together with crushing force. The protractor muscles carry the jaws forward (with a rotary or rocking movement on the muscular pads) against an object of attack, the lower jaw acting with its teeth as a hook, while the powerful retractor muscles of the upper jaw bring its toothed blade with a shearing motion between the ventral teeth. Thus is constituted an efficient pruning apparatus, the chief purpose of which is, I believe, the clipping off of branchial filaments of the crayfish host, from which the blood is then drawn. They are probably also used for moving down the colonial infusorians which cluster along the borders of the branchial chamber, and remains of which are frequently seen with diatoms, *etc.*, mixed with crayfish blood in the stomachs of worms examined.

The jaws mark the beginning of the pharyngeal region, which extends to the œsophagus in the anterior part of the first post-cephalic somite (Fig. 9). The region is distinguished by the great development of muscular tissue, and doubtless functions in the capacity of a suction bulb to increase the flow of blood from the wounded tissues of the host. The structure is similar throughout. A delicate cuticle lines its lumen, thinning out and disappearing where the transition from pharyngeal to œsophageal regions takes place. The epithelium is characteristic. As has been described above, cell boundaries are seldom clearly distinguishable, but the deeply staining protoplasm, with its contained nuclei, is crowded, with the exception of a delicate continuous layer, from its normal position at the surface, into irregular processes, which fit plug-like between

the ends of the radial muscle fibres (Fig. 11, *ep*). If the epithelium could be completely isolated, it would present somewhat the appearance, without the regularity of pattern, of those ingenious rubber change mats which are in such frequent use on the show-cases of our stores.

Immediately external to the epithelium and highly developed is a continuous layer of circular muscles. The fibres have a very regular arrangement (Fig. 9, *ep*), are completely tubular, and increase in size toward the posterior end of the head, where they pile up into several layers and pass into the posterior cephalic septum, behind which they rapidly diminish into the delicate muscular coat of the œsophagus.

Radiating on all sides from the pharyngeal walls, traversing the head cavity and passing into its outer walls, are numerous large isolated muscle fibres (Figs. 9 and 11, *vm*). These originate externally from the longitudinal muscles, become somewhat constricted within the head cavity, and break up at their inner ends into numerous branches which spread and straddle the circular fibres, passing between them and penetrating the pharyngeal epithelium as described above. They are among the largest muscular fibres of the body, reaching a diameter of .025 mm. The central granular protoplasm is well marked, and contains a conspicuous nucleus, which may, however, lie on the exterior of the fibrillated protoplasm (Fig. 11, *vm'*). A pair of small, berry-shaped glands are attached, one on each side, to the lateral angles of the pharynx, just behind the jaw pads. They are partially indicated in Fig. 10, *g*.

The œsophagus is short, and its posterior limit rather ill defined. It is characterized by a sudden diminution of muscular tissue in the walls of the digestive tract, which takes place after penetrating the posterior cephalic septum and entering the first body somite, by a change in the character of its lining epithelium, and by the absence of chloragogue cells, which distinguish the intestine. The alimentary canal begins to increase in diameter within the body segments, and exhibits a slight sacculation in the second. In this region the lining epithelium is a single stratum of columnar cells, with freely rounded and often bulbous, projecting ends (Fig. 7, *oe*). No

cuticle nor cilia have been detected in this region. The muscular coat of the œsophagus is principally made up of thin, transparent, non-granular fibres, forming a sheet which blends with each successive muscular septum, and is continuous without much change throughout the remainder of the alimentary tract. Circular muscles are distinguished as delicate rings on the inner face of the longitudinal sheet, but become much more conspicuous at the septal constrictions, where they derive additional fibres from the septa themselves.

The modification of the peritoneum to form chloragogue cells begins in the third or second somite, behind which the intestine is enveloped in a continuous layer to the seventh somite, where they are absent (or sometimes sparingly scattered among the peritoneal cells by which they are replaced), but reappear in the eighth and ninth somites (Figs. 5, 12, 13, and 15). In surface views of fresh material the chloragogue cells appear as a mosaic of large polygonal cells, with straight closely fitted edges, possessing a clear central nucleus, and cytoplasm of a greenish brown color, due to the presence of numerous large granules and minute globules. In sections they appear more or less flattened, or prominently bulging, according to their position and the degree of contraction of the intestine. In the region of the dorsal blood vessel they become elongated and arch over its walls. From their flattened or concave bases protoplasmic filaments and columns arise, which cross the peri-enteric sinus and bind the chloragogue cells and the epithelium more firmly together. By the increase of such strands in size and number the sinus is broken up anteriorly and posteriorly into plexuses. The granules with which the protoplasm is filled are stained deeply by most dyes; and the clear globules above mentioned blacken with osmic acid, while unaffected by ordinary stains, and dissolve in chloroform, *etc.*, appearing in sections as spaces. Their evident fatty nature leads me to regard the chloragogue cells as absorptive, while, on the other hand, their relation to the peritoneal corpuscles renders an excretory function probable. There seems, then, little choice between the two opposing views which have been brought forward, both probably being in part correct.

In the seventh or ovarian somite the absence of chloragogue cells (Fig. 15) permits the maturing ova to come into close contact with the walls of the blood sinus, which they envelop—a nutritive arrangement of importance. True chloragogue cells are again absent in the tenth segment on the rectum, which is covered by a layer of cubical peritoneal cells (Fig. 5). In the region of the anus circular muscle fibres increase to form a sphincter.

The epithelium of the intestine presents the same general characters as that of the œsophagus, but becomes more flattened (Fig. 12 and 13), especially in the saccular enlargements, while at the constrictions the elongated cells project into the lumen to form valve-like outgrowths (Figs. 5 and 13). It is ciliated only in the posterior part (Fig. 5).

Vascular system.—The vascular system differs in no important respect from the description given of Branchiobdella by Dorner and amended by Voigt. The peri-enteric blood sinus, to which Voigt first specially called attention in Branchiobdella, is highly developed in the present species, in which it exists as a continuous space between the muscular and epithelial coats of the intestine, extending from the third to the eighth somites inclusive, and breaking up at each end into a system of passages and lacunae having a retiform arrangement (Fig. 1). The sinus has an average depth of .005 mm.; and is without true walls other than the intestinal coats between which it lies. It is crossed by numerous protoplasmic strands and columns which bind its walls together, and remind one of the stalactites and columns of a limestone cave. These become larger and more frequent toward the ends of the sinus, which they finally interrupt so much as to convert it into the terminal plexuses mentioned. In Fig. 5, drawn from a specimen in which the sinus continued complete even into the ninth somite, these strands are well shown owing to the absence of any considerable quantity of blood, which often stains deeply and obscures not only the strands but the muscular coat as well. Enlarged chambers exist here and there in the course of the sinus; and continuous enlargements extend along the dorsal and ventral regions. The ventral or sub-

intestinal enlargement remains throughout its entire length strictly a part of the sinus, with which it is in continuous communication; but the dorsal one, which has a similar relation posteriorly, begins in the fifth somite to have the character of a distinct vessel, which has more or less complete walls and rises above the level of the sinus, from which it may be entirely free for a short distance, but again open into it through a cleft (Fig. 12). Perforating the septum 4-5, from which its muscular walls receive accessions, it becomes a distinct muscular tube, still imbedded amongst the chloragogue cells, and, increasing in diameter, passes obliquely down the right side of the intestine, with which it loses all connection in the third somite, and becomes a regularly pulsatile tube—the so-called heart. The heart thus formed is thrown into a conspicuous loop, which accommodates itself to changes in the animal's length. It lies to the right of the oesophagus, and in the first somite rises once more to a dorsal position and gives off the fifth pair of vascular arches; anterior to which it continues into the head as the dorsal trunk.

At the place of origin of the heart from the blood sinus certain chloragogue cells extend into its lumen, and give rise to a remarkable chain of valve cells, which retain all of the visible characters of the chloragogue cells. This rod is chiefly confined to the ventral wall of the heart, to which it is more or less closely bound. The inter-cellular space described by Voigt is well marked in this species, and may be seen to increase and decrease in size with the expansion and contraction of the heart. Occasionally in sections (Fig. 14, *b*) the cells form only a thin wall around a conspicuous lumen. In some cases also there are indications of a special muscular layer surrounding the cells (Fig. 14). They are also attached here and there to the walls of the heart by delicate threads (Fig. 7, *h*). The rod is continuous throughout the greater part of the heart and may even extend into the dorsal vessel of the head (Fig. 9). Well fitted to serve as valves, the peculiar arrangement of the cells permits them to act also in the direct propulsion of the blood. The cells present more flattened surfaces anteriorly, and more sloping ones behind, and swinging forward as the

wave of contraction passes along the heart walls, push the blood along, and then return without offering much resistance, and complete another oscillation before coming to rest. As the wave of contraction passes forward they close the lumen of the heart more effectually than would be possible for the muscular walls alone, and add greatly to the efficiency of the organ.

The dorsal vessel of the head is very much smaller than the heart, and as the posterior portion is also elastic and somewhat contractile, the flow of blood is doubtless nearly or quite continuous, although the heart contracts intermittently. From it three pairs of conspicuous but delicate lateral trunks arise, corresponding to the three posterior cephalic annuli, and wind among the muscles around the pharynx to join the sub-neural vessel. The most anterior of these three blood arches is closely associated with the circum-oesophageal nerve connective. Finally the dorsal vessel terminates, after passing beneath the supra-oesophageal ganglion, in a pair of trunks which arch through the lips close to the salivary gland, and meet ventrally to constitute the supra-neural blood vessel (Figs. 1 and 17), which is soon increased by the accession of the second, third, and fourth pairs of cephalic blood arches (Fig. 16), and, in the first body somite, by the fifth pair of arches. Throughout its entire length the supra-neural vessel lies in contact with the dorsal side of the nerve cord, and passes between the ganglia of each pair in a deep groove (Figs. 1, 7, 11, *etc.*). In the seventh somite it gives off a pair of large ovarian vascular arches, which empty into the dorsal enlargement of the peri-enteric sinus (Fig. 1). The supra-neural vessel terminates in the tenth somite in a pair of large trunks, which arch around the intestine, and pass forward to empty into the dorsal region of the peri-enteric sinus, thus forming the beginning of the dorsal enlargement, which here receives the dorsal ends of the plexus of blood passages (Figs. 1 and 5). Of the seven pairs of lateral arches mentioned, a labial and three cephalic pairs, in the pharyngeal region, arise from the anterior prolongation of the heart; an oesophageal pair, which owing to their great length are often looped into the succeeding somite, arise from the anterior end of the heart itself; a

large pair of ovarian arches, which are more or less imbedded in the maturing ova, lie in the posterior part of the seventh somite ; and the seventh pair, which are the largest of all, posteriorly connect the supra-neural vessel with the peri-enteric sinus. The walls of the supra-neural and lateral vessels are very delicate and non-contractile. At wide intervals, nuclei, which resemble those of the peritoneal cells, may be detected, but I have found no traces of cell boundaries.

Nervous system.—The nervous system does not differ in any important particular from the description given by Dorner for *Branchiobdella*. It consists of a pair of supra-oesophageal ganglia lying just posterior to the dorsal jaw pad. The two ganglia of this pair are united across the median line by a cord of nerve cells and a fibrous commissure ; and each bears well marked posterior lobes, which are themselves divided into larger external and smaller internal parts, and are connected with the main ganglion by three strands of nervous matter (Fig. 17). The vascular arches of the second pair pass along the groove between the anterior and posterior divisions of the ganglia, and follow the connectives about to be described. The circum-oesophageal connectives are thick strands of nerve fibres, with a partial covering of nerve cells which extend from the ganglia particularly along their posterior and anterior faces. They pass around the pharynx, and, just before meeting on the ventral side, each bears a bi-lobed pedicled ganglion (Figs. 1 and 16), and after uniting to form the ventral nerve cord, two succeeding pairs of similar but larger ganglia ; making in all four pairs of double ganglia within the limits of the head (Figs. 1 and 16). The nerve cord, which consists of two distinct halves throughout its length, enlarges at the ganglia and shrinks in the inter-ganglionic intervals. Numerous nerve fibres arise from the circum-oesophageal connectives and the superior ganglia, and pass to the peristomial region. In *B. philadelphicus* these can be readily traced by the use of methylene blue to the circum-oral hairs, and especially to the oral papillae.

In the post-cephalic somites a ventral chain of eight pairs of bi-lobed ganglia is succeeded by a posterior ganglionic mass,

representing three pairs of coalesced ganglia which successively diminish in size toward the posterior end (Fig. 1). The anterior eight are situated in the major annuli of the corresponding number of somites. They are of equal size and similar form ; the posterior lobe of each being slightly smaller than the anterior. The fifth and sixth are slightly displaced to the right of the middle line, respectively by the spermatheca and the atrium, especially the sixth, which is also turned on edge and bound to the muscular wall of the copulatory bursa. Dorsally all of these ganglia rise above the level of the nerve cord (Figs. 7, 11, and 18) to which they are broadly attached ; forming a series of grooves in which the supra-neural vessel is accommodated. Three pairs of nerves spring from the region of each pair of ganglia, one from each end and one from the transverse constriction (Fig. 18). These supply the body walls, and are readily traced between the two layers of muscles, splitting up as they proceed. The first and second nerves supply the major annulus alone, the third (posterior) divides, one branch passing to the major, the other to the minor annulus. The posterior mass is related to the caudal concentration of somites for the support of the sucker, and lies posterior to the septum 8-9. It does not differ in minute structure from those anterior. The anterior ganglion of the mass is larger and the posterior smaller than the isolated pairs of ganglia. Several lateral nerves have their origin here, and the cord terminates in a brush of smaller nerves which supply the numerous glands and muscles of the region. Long narrow fissures appear along the middle line of the nerve cord at intervals for its entire length, and frequently penetrate completely through, separating the two halves of the cord.

A thin sheath which encloses the nerve cord for its entire length and includes the supra-neural vessel, appears to be muscular.

Besides the smaller ganglion cells which make up the bulk of all of the ganglia, each ganglion possesses a small basal group of larger ones (Fig. 18). No visceral nervous system has been detected ; nor any of the large isolated ganglion cells figured by Voigt in connection with the acetabular glands.

Reproductive system. — The reproductive organs of *Bdello-drilus* differ in certain important respects from those of *Branchiobdella*, as described by Dorner, Keferstein, Voigt, Vejvodsky, *etc.*; but nevertheless there is a close general resemblance between the two. Resembling its allies, *Bdello-drilus* is hermaphroditic, and possesses accessory sexual structures admirably fitted to the process of reciprocal fertilization, which, no doubt, occurs during copulation. The general arrangement of the reproductive organs is shown in Fig. 1.

The male organs occupy the greater part of the peri-enteric cavities of somites five and six. They consist of two pairs of testes, and two pairs of vasa deferentia, having a common opening to the exterior by means of a complex unpaired atrium, which is differentiated into a glandular sperm sac, a muscular sperm sac, an eversible penis, and a muscular copulatory bursa, with its associated glands. The testes proper can be distinguished satisfactorily only in the young, in which, if examined shortly after hatching (or after removal from the cocoon just previous to that event), they are easily detected in entire worms or in sections as small groups of rounded nucleated cells, attached near the floor of the body on each side of the posterior faces of septa 4-5 and 5-6. Their origin from the peritoneal lining of the coelom is evident upon the examination of longitudinal sections of several successive stages of development. The first steps in the development of spermatozoa begin before the worm has nearly reached full size, and proceed continuously; the various stages floating freely in the coelom, in which they complete their development. In the mature worm the cavities of the fifth and sixth post-cephalic somites are filled with spermatozoa in various stages of development, while the testes proper have become much reduced and inconspicuous. The details of this process have been admirably worked out and described by Voigt; and it need only be added that what observations the writer has made are in accordance with his account.

The male efferent ducts (Figs 1, 19, and 20) correspond in number to the testes, and their mouths open into the same somites. The mouths of the anterior pair lie far forward in

somite five, both being usually displaced to the left side by the spermatheca, which occupies the right of the same somite. Penetrating the septum 5-6 close together near its ventral attachment, they unite, and proceed as an unpaired duct to the copulatory bursa, to the anterior walls of which it is attached, and then turns sharply upward to empty into the middle of the glandular sperm reservoir. The posterior pair, lying entirely in somite six, are shorter and more symmetrically arranged. Their free ends are attached to the anterior face of the septum 6-7, from which their mouths turn forward. The common duct into which they unite is slightly attached to the bursa, and empties into the sperm sac at the same place as the anterior (Fig. 19). These vasa deferentia are rather broad thick-walled tubes with narrow lumens. They are lined by a naked cubical epithelium, which becomes more columnar and acquires cilia a short distance from the free end, which presents no funnel-like expansion. (In a single preparation, out of a great many examined, all four vasa deferentia possessed expanded mouths.) The epithelium is covered by an outer layer of narrow elongated cells, which are placed transversely, and form a complete investment around the duct, except of the ciliated cells, which extend freely beyond this covering (Fig. 25). These cells are undoubtedly muscular, and have the same structure as the larger muscle fibres which similarly invest the walls of the spermatheca (Fig. 21). In living animals subjected to pressure a faint and irregular wave of contraction may sometimes be seen to travel along the walls of a vas deferens toward the atrium; and such peristalsis doubtless serves to impel the spermatozoa along the non-ciliated passages.

The atrium (Figs. 19 and 20) or enlarged terminal portion of the male efferent apparatus, is divided into two distinct portions, the internal glandular spermatic vesicle, which belongs to the vasa deferentia, and serves to collect and retain the spermatozoa; and a prominent muscular-walled region which functions as a copulatory organ, and is derived from an invagination of the body walls. The former (Figs. 19 and 20, *sz*) is a short curved sac, somewhat enlarged at the free end and tapering toward the other, where it suddenly contracts in

diameter before emptying into the anterior side of the enlarged inner end (muscular sperm sac) of the penis. The glandular sperm sac lies in contact with the anterior side of the penis sheath, where it is held by the vasa deferentia (Fig. 19). At about the middle of its length it presents a slight ventral enlargement which receives the vasa deferentia, beyond which it curves abruptly upward. The thick glandular walls are made up principally of deeply-staining columnar or irregularly pyramidal cells, with distinct basal nuclei, and reticulated granular protoplasm (Figs. 22 and 23). At the place of communication with the muscular sperm sac these cells perforate the muscular coat of the latter, and become continuous with its lining epithelium (Fig. 23). No cilia are present in the sperm sac.

The copulatory region of the atrium is formed by a single invagination of the entire thickness of the body wall; the epidermis becoming the epithelial lining, and the longitudinal and circular muscle coats, respectively, the longitudinal and circular muscle coats of the atrium. Proximally the invagination becomes expanded into a conspicuous sub-spherical copulatory bursa (Figs. 19 and 20), the posterior dorsal portion of which is continued into the sub-cylindrical penis sheath (Fig. 19). The thick muscular walls of the bursa are principally derived from the longitudinal muscles of the body. These continue their longitudinal direction over the bursa and penis sheath, increasing in thickness on the anterior wall of the former, while on the latter only a single layer of closely appressed fibres is developed. They cease entirely at a constriction which marks the upper end of the penis and the beginning of the muscular sperm sac (Figs. 19 and 20). At the point where the bursa passes into the penis sheath a reflection of a portion of the longitudinal fibres (which pushes the epithelium before it) into the cavity of the former, gives rise to the basal portion of the penis, which, when retracted, projects as a conical process downward and forward into a space between the bursal glands (Figs. 19, 20, and 26).

The circular muscle fibres become in the walls of the bursa quite distinct from the epithelium and more closely associated

with the longitudinal muscles. Around the mouth of the bursa they form a sphincter, but elsewhere a thinner layer one fibre deep. This is interrupted in the upper part of the copulatory bursa by the enormous enlargement of two lateral groups of epithelial cells to form the bursal glands, which push between the two muscular layers and occupy what corresponds to the spaces between the longitudinal and circular muscles of the body walls, bulging the side walls of the bursa outwards, so that the transverse diameter is fully one-half greater than the antero-posterior (Figs. 19, 20, 24, and 26). The circular muscles thus lie entirely within the bursal glands, between these and the lumen of the bursa.

In the penis sheath (Figs. 19 and 20) the circular fibres become still further distinct from the epithelium, and closely connected with the longitudinal muscle coat; leaving a nearly empty space all around, between the epithelium and the circular muscles. Numerous small muscular slips, from the circular fibres, traverse this sub-epithelial space and insert among the epithelial cells. For the sake of clearness these have been omitted in the partly diagrammatic Figs. 19 and 20. These fibrils, which must be capable of great extension, are an important factor in the eversion and retraction of the penis. At the constriction of the penis sheath above mentioned the sub-epithelial space ceases (Figs. 19 and 20), and the circular muscles come in direct contact once more with the epithelial cells. They extend together beyond the limit of the longitudinal muscles, and expanding, form a prominent vesicle (Figs. 19 and 20 *sv'*) which receives the glandular sperm sac, and into the cavity of which the lumen of the penis expands. The muscle fibres of the atrium (Figs. 24 and 26) possess rather elongated nuclei, and differ somewhat in minute structure from those of the body walls, being rather a transition form between these and such as cover the spermatheca. The fusiform shape which Fig. 26 shows is due to oblique section.

The epithelial portion of the invagination is derived from a cellular zone of the epidermis, and undergoes no change throughout the greater part of the bursa, except in the dorsal region, where a pair of conspicuous glands are developed, one

on each side of the space in which the end of the penis lies (Figs. 20, 24, and 26, *g*). The gland cells are arranged in an almost spherical group around a deep hilum, or sinus, which serves as a duct, and opens directly into the cavity of the bursa. The cells are pyramidal, with broad, deeply staining bases containing the nuclei; and elongated necks which are highly granular, but stain less deeply, and converge toward the sinus, in which the cuticle is interrupted (Fig. 26). The presence of these glands complicates the shape of the cavity of the bursa. Ventrally (below the glands) it is transversely extended, and, as the glands stand out from the posterior wall, antero-posteriorly constricted; higher up (between the glands) it is narrowed in its transverse dimension, and extended antero-posteriorly; dorsally it expands, but is much encroached upon by the projecting end of the penis. Figs. 19, 20, and 24 will make this clear.

Over the free projecting end of the penis the epithelium is, of course, reflected, and then continues with its cuticular covering as the lining and eversible portion of that organ. Here the cells become rather smaller, but regain their size in the muscular sperm sac, into which the cuticle also continues. This penis epithelium is, as it were, much too long for its muscular sheath, and is consequently thrown into folds or slightly spiral coils within the sub-epithelial space (Figs. 19 and 20, *pe*), the narrow lumen appearing as a conspicuous wavy double contour. In protrusion these waves are straightened; partly pulled out by the contraction of the minute muscular fibrils, partly squeezed out by the pressure of the circular muscle coat, and partly by the shortening of the whole organ, owing to the contraction of the longitudinal muscles. By the combined action of these forces the epithelium is everted, and projects from the external opening of the bursa as a double-walled tube, slightly bulbous at its free end, and covered with a smooth cuticle. Whether or not the bursa itself is everted naturally in this species is a question. By careful application of pressure while under the microscope I have been able to force it through the external opening; but it always carries the sixth nerve ganglion with it under such circumstances;

and, although naturally everted penes are not difficult to observe, the bursa is never displaced. The everted penis in such cases is quite long enough to be functionally useful. In *B. philadelphicus*, on the other hand, the penis and its sheath are united into a solid piece, incapable of eversion, and here the whole bursa, which has quite a different structure, is everted naturally and carries the short projecting end of the penis with it. The inner surface of the bursa is smooth in *B. illuminatus*, and shows no trace of the elongated and more or less branched papillae which are present in certain other species.

The ovaries (Fig. 1) consist of a pair of cell masses attached one on each side to the septum 6-7, and hanging into the coelom of the seventh somite. In immature individuals they have the arrangement described by Voigt for *Branchiobdella*, but are more slender, and are borne on longer muscular pedicles. In the mature worm the ovaries have become much more bulky and of irregular shape (Fig. 1). Their mass is made up of young ova (Fig. 27) of nearly uniform size. The immature ova are spherical, with a large, clear nucleus and distinct nucleolus. A transparent stroma in which the ova are imbedded appears to be present, and the ova themselves are arranged in irregular rows, showing the manner in which they have proliferated and pushed out from the germinal epithelium. Among the ova of the peripheral part of the mass a few enlarged ones in the early stages of maturation are always present, and two or three large irregular ones nearing maturity. The latter have usually broken loose from the ovary, and, as irregular masses, conform to the shape of the perivisceral cavity, which they largely fill, closely enveloping the intestine. A clear nucleus may always be detected in living examples, in which the white opaque ova are very conspicuous. In such ova the nuclei have become very much enlarged and somewhat vesicular. The entire cytoplasm is filled with minute rounded yolk granules (Fig. 28, *o*), among which a few deeply staining specks are scattered. During the period of maturation the ova appear to receive nourishment directly from the peri-enteric blood sinus, with the thin wall of which they lie directly in contact. The pair of vascular arches,

around which the ova frequently wrap, serve the same purpose. Large coarsely granular masses, which Voigt regards as fatty degenerated ova, are frequently present, and appear to be absorbed by the maturing ova.

No free oviducts are present, the mature ova escaping by a pair of simple ovipores (Fig. 28), which are ridiculously small as compared to the mature ova, though capable of considerable distention, and perforate the body walls in the constriction between the major and minor annuli of the seventh somite, each in the ventral octant of its respective side. The lining epithelium resembles that of the copulatory bursa, *etc.*, and spreads out on all sides of the internal aperture as a flaring lip (Fig. 28). Cilia are wanting, and the ova are extruded by pressure of the body muscles, a process which may be successfully simulated by artificial means. The ova are very plastic, but at once assume a rounded form in the water. Fig. 28 is taken from a preparation of *B. philadelphicus*, as the section passed through a more favorable plane for representation than any of *B. illuminatus*. No essential difference, however, is observable between the two species. Note, however, the much larger size of the longitudinal muscle fibres and the smaller size of the epidermal nuclei in *B. philadelphicus*. Part of an ovum is shown at *a* lying opposite to the mouth of the ovipore.

As in *Branchiobdella*, the spermatheca is unpaired; but in the present species has a very characteristic form (Figs. 1 and 29). The proximal portion opening on the ventral surface of the fifth somite is sub-cylindrical, but expanded just within the mouth by large anterior and posterior glands. Dorsally by the side of the intestine it splits into a pair of clavate sacs, of which the external (Fig. 29, *o*) is the larger, and is often thrown into a coil. The internal slightly smaller sac (*i*) has usually the appearance of a lateral branch, being more displaced from the axis of the organ than the outer. The lining epithelium has also a more glandular appearance (Fig. 30). In living animals both divisions show slight terminal enlargements, and, owing to contractions of the muscular coat, irregularities in diameter.

Around the mouth of the spermatheca the epidermal cells become more columnar, producing the slight swelling of the lips (Fig. 29, *cp*). Immediately within the mouth the transparent (almost vitreous-appearing) ductules of the spermathecal glands empty. These glands are hemispherical aggregations of small pyramidal gland cells (Fig. 29).

Above this point the epithelium maintains a nearly uniform character, becoming somewhat deeper and more glandular in the smaller of the two branches. The cells are large and deep, and have polygonal basal outlines; and good preparations show a distinct division of the protoplasm into a basal more opaque and an apical (next to the lumen) clear zone (Fig. 30), the free ends of the cells being often very indistinctly bounded. The large central nucleus exhibits a distinct chromatin network (Fig. 30).

Two layers of muscular fibres invest the undivided portion. Of these the outer longitudinal extends with a slight twist as a thin transparent sheet a single fibre deep, over the glands nearly to the point of bifurcation, where it fades out (Figs. 29 and 32). Each fibre possesses an ellipsoidal nucleus having its longer diameter directed longitudinally; and clear, almost homogeneous, but deeply staining cytoplasm (Fig. 32). The powerful circular layer is more extensively developed. Beginning above the glands, it forms a continuous layer of remarkable encircling fibres, mostly arranged transversely to the long diameter of the organ, but usually more or less spirally on the blind end of the smaller division, an arrangement which is sometimes even more regular and conspicuous than in the example figured (Fig. 29). Owing to the thick rounded form of these cells (Fig. 30), and the somewhat irregular manner of their disposition, the surface of the organ is thrown into rounded ridges (Fig. 30), which, during life, are rendered still more prominent by the irregular contraction of the muscle fibres. The fibres of the circular muscle layer resemble those of the longitudinal layer in structure and appearance; the cytoplasm is even more homogeneous and clear (Fig. 32). Many of the fibres pass almost entirely around the spermathecal walls. They taper toward the ends and overlap one another, as shown in Figs. 29 and 32.

Excretory system. — The number of nephridia is reduced, as in *Branchiobdella* (Dorner, Vejdovsky, *etc.*), to two pairs. Of these, one pair is located symmetrically entirely within the eighth somite (may communicate with the seventh), opening to the exterior by small non-pulsating vesicles on the dorsal surface of the body between the eighth and ninth somites. In structure they are similar to the anterior ones (Fig. 34).

The anterior pair alternate in position, one occupying the body cavity to the left of the alimentary canal in the second and third somites, and extending forward to the right side of the first; the other lying mostly within the fourth somite, of which its most conspicuous portion occupies the right side. The cellular masses into which the nephridial tubules plunge (compare Dorner) are darkly colored and conspicuous in the living animal, that belonging to the anterior nephridium being located on the left side of the third somite, that of the more posterior to the animal's right in the fourth somite.

The somewhat complicated course of the tubules has not been fully unravelled in detail, so that the present account will be confined to the statement of a few clearly ascertained facts. The anterior pair of nephridia have a common external opening through a median vesicle on the dorsum of the third major annulus. This is formed from an invagination of the epidermis, which lines it, and a covering of muscle fibres (Fig. 33). An internal proliferation of its lining cells forms a nearly complete valve-like diaphragm, which divides the interior into an anterior larger compartment opening to the exterior by a pore, and a posterior smaller one which receives the terminal portions of the nephridial tubules, and communicates with the anterior through an opening in the diaphragm. The terminal portions of the tubules, which are of large size, pass from the vesicle transversely around the body, one to the right, the other to the left, between the two muscular layers; and, entering the body cavity close to its floor, diminish decidedly in diameter (Fig. 35), and then pass into a long tubule which is arranged in a complicated series of long loops, which pass over and around the intestine, and, in the case of the left nephridium, reach far forward into the first somite, and, of the right

nephridium, extend transversely across the intestine, in contact with the anterior face of the septum 4-5. The tubules of this region are more or less enveloped by greatly enlarged peritoneal cells, which enwrap the groups of tubules, and become more or less fused with their fellows into a continuous protoplasmic mass, though many stand out freely from this mass, and, in the living worm, are swept backward and forward with currents of the coelomic fluid produced by the heart's pulsations, *etc.* Even in the mass of cells the boundaries are often perfectly evident, and the cells may even be widely separated (Figs. 36 and 37). Their central protoplasm is arranged in a coarse network, the meshes of which become smaller, and finally almost indistinguishable at the periphery. The nuclei are large and distinctly nucleolated (Figs. 36 and 37).

The tubules themselves, including the terminal portions quite to the point of union with the vesicle, are made up of tubular cells joined end to end in a continuous chain, with a continuous central lumen. This lumen exhibits slight irregularities in diameter, and may wind more or less from side to side within the substance of the cell. Where two cells are joined the lumen almost invariably develops two, three, or four short cæca (Fig. 35), which sometimes reach almost to the surface of the cell, and may run together more or less to form an irregular chamber. Midway between two of these nodal points the nucleus may always be seen lying close to the lumen. At the point where the tubule leaves the body cavity a curious arrangement of the lumen exists. Here there is developed either an irregular chamber partly divided by columns or islands of protoplasm, or else, and more frequently, an irregular branching and looping of the lumen, one form of which is shown in Fig. 35. The walls of the living tubules of this region exhibit evident radial striations passing as dark lines from the lumen to the external surface of the cells (Fig. 35). Sections across the four tubules, the irregular windings of which make up the greater part of the terminal loops of the nephridia, especially at points where their approximation enables us to readily compare their appearance, show a slight difference in the structure of their cell walls. In the case of

one pair (the two limbs of a loop, Fig. 36, *n*), the protoplasm is more uniformly distributed, and the granules show a decided tendency to become arranged in lines radiating from the lumen, as seen also in living cells; while the protoplasm of the remaining pair (forming another loop) is more or less reticulated and less deeply stained (Fig. 36).

The system of simple tubules is partly concealed in the dark cellular mass mentioned above, where it becomes connected with a series of intra-cellular plexuses—not mere lateral branches of the main lumen, as Bourne has described in certain leeches, but a regular series of actual interruptions of the simple lumen.¹ The course of the tubules within this mass is very irregular and tortuous. Turning abruptly on itself at frequent and regular intervals, the tubule expands into irregularly rounded nodules of about three times the ordinary diameter. Within the highly granular protoplasm of these swellings the lumen breaks up into a complex system of irregularly branching and communicating canals, all of which converge at the opposite point of the nodule to empty into the next section of the simple tubule (Fig. 39), so that in this region there is a regular alternation of short lengths of simple ciliated canals and irregular plexuses of non-ciliate anastomosing canals. The latter may be aptly compared to the communicating passages which termites excavate in the trunks of trees; but the capacity of the passages relatively to the amount of solid substance between and around them is less in the structure here described.

Nuclei are present both in the simple segments of the tubules and in the plexus cells (Figs. 38 and 39). Cilia are distributed throughout a considerable portion of the simple tubules, and in the simple canals connecting successive plexuses (Fig. 39); and are absent from plexus passages and in the terminal segment of the tubule (Fig. 35). No nephrostomes have yet been detected in this species; but Fig. 40 represents one of *B. philadelphicus*, which has nephridia of similar structure.

¹ See also Eisen's paper on the remarkable nephro-plexuses of *Deltania* and *Argilophilus* which I have read since writing the above. *Mem. Cal. Acad. Sci.* Vol. II., No. 3. 1894.

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DESCRIPTIONS OF FIGURES.

Unless specially stated otherwise, all drawings have been made upon camera lucida tracings. With the exception of Figs. 28 and 40, which represent parts of *B. philadelphicus*, all figures are drawn from preparations of *B. illuminatus*.

EXPLANATION OF PLATE XXVIII.

FIG. 1. A diagram of the general anatomy of *Bdellodrilus illuminatus* showing the principal organs, enlarged about eighty diameters. The entire vascular system is colored; the principal trunks and the dorsal and ventral enlargements of the sinus being a darker, and the greater extent of the sinus a paler tint of the same color. The post-cephalic somites are numbered 1 to 11; the four annuli anterior to 1 constitute the head.

Alimentary canal. *P*, pharynx; *æ*, oesophagus; *S*, two of the stomachic enlargement; *a*, anus. In the seventh somite the intestine is hidden by the ovaries and maturing ova.

Vascular system. *H*, *H*, heart; *va*¹⁻⁷, the seven pairs of vascular arches, of which four are in the head, and one each in the 1st, 7th, and 9th post-cephalic somites; *sn*, *sn*, the supra-neural blood vessel; *pa*, the anterior, and *pp*, the posterior peri-enteric plexuses which terminate the sinus; *s*, sinus, the dorsal and ventral enlargements are indicated by their deeper color.

Nervous system. *sg*, principal, and *sg*¹, the accessory supra-oesophageal ganglion; *cc*, the circum oesophageal connective; *cg*¹⁻³, the three pairs of double ventral cephalic ganglia; *g*¹⁻⁸, the ganglia of the first eight post-cephalic somites; *gn*, the posterior ganglionic mass.

Reproductive organs. *t*¹ and *t*², testes, represented as they appear in the immature worm; *vd*¹ and *vd*², the anterior and posterior pairs of vasa deferentia; ♂, the male pore; *bm*, *bm*, the muscular walls of the copulatory bursa; *bg*, the bursal glands; *sv*, the glandular, and *sv*¹, the muscular sperm sac; *Pe*, penis; *Ov*, ovary; *O*₁, *O*₁, nearly mature ova; *O*¹, smaller, but developing ova; ♀, female pore; *sp*, *sp*¹, and *sp*², the undivided, the outer, and the inner divisions of the spermatheca, respectively.

General *nv*, the common vesicle of the anterior nephridia; *nt*, the terminal portion of the nephridial tubule of the right side; *lg*¹⁻², lateral glands, dorsal and ventral points of outlet shown for all, complete glands outlined in somites 2, 4, and 8, the last a divided one.

FIG. 2. A transverse section of a portion of the epidermis from the dorsum of the 5th somite. Kleinenberg's haematoxylin. × 500. Cellular zone to the right, non-cellular to the left of the figure. *cc*, cuticle; *sl*, striated lamina; *ec*, groups of nucleated cells; *sa*, cleft or space in the protoplasm, *d*, *d*, dark column; *cm*, circular muscle fibres; *n*, nucleus.

FIG. 3. Tangential section through region of Fig. 2, lettering the same. × 500.

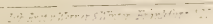
FIG. 4. Longitudinal section through a portion of the clitellum (dorsum of sixth somite). Alum cochineal. $\times 195$. *c, c*, epidermal cells; *g, g*, glands; *lm*, longitudinal muscle fibres; *cm*, circular muscle fibres.

FIG. 5. Partial reconstruction, from camera drawings of successive longitudinal sections, of a median projection of the posterior end of the body. Alum cochineal. $\times 195$. The body walls are shown in outline. *ag, ag*, acetabular glands; *d, d, d*, groups of ductules which are distributed in the hypodermis; *g⁸*, eighth ganglion; *gm*, posterior ganglionic mass; *n*, terminal bundle of nerves; *cc*, chloragogue cells; *ec*, ciliated epithelium of intestine; *bs*, blood sinus; *a*, anus; *va'*, terminal vascular arch.

FIG. 6. Diagram of a lateral gland, shown as though partly cut open to exhibit internal structure.

FIG. 7. Transverse section through the major annulus of the 1st somite. Borax carmine. $\times 195$. *lg*, lateral glands; *gv, gv*, ventral, and *gd, gd*, dorsal openings of ductules; *h*, heart; *vc*, the internal chain of valve cells; *a*, œsophagus; *nc*, nerve cord; *sn*, supra-neural blood vessel; *g*, ganglion; *va*, portion of fifth vascular arch; *lm*, longitudinal muscle fibres; *hy*, hypodermis.

FIG. 8. Cross-sections of four forms of longitudinal muscle fibres. Haem. $\times 500$. 2d somite.





EXPLANATION OF PLATE XXIX.

FIG. 9. A reconstruction, from camera drawings, of a median plane projection of the head. *hy*, hypodermis; *og*, oral glands; *sg*, salivary (?) gland; *ag*, adhesive gland; *dj*, dorsal, and *vj*, ventral jaw; *dm*, dorsal, and *vm*, ventral muscular pads; *dpm*, dorsal, and *vpm*, ventral protractor muscles; *drm* (foremost index line) dorsal, and *vrn*, ventral retractor muscles; *lm*, longitudinal muscle fibres; *rm*, radial muscle fibres; *cm*, circular muscle fibres; *ep*, pharyngeal epithelium; *pc*, cuticle of pharynx; *sga*, supraoesophageal ganglion; *nc*, ventral nerve cord; *g*¹, first post-cephalic ganglion; *dv*, dorsal blood vessel; *sv*, supra-neural blood vessel.

FIG. 10. Transverse section of the head in the region of the jaws. Kleinenberg's haematoxylin. $\times 195$. Lettering as in Fig. 9. *g*, *g*, pharyngeal glands.

FIG. 11. Transverse section through the middle region of the head of a larger individual than 9 and 10. Klein. haem. $\times 195$. Lettering as in Figs. 9 and 10. *d*, *d*, groups of ductules passing from head glands to the lower lip; *rm*¹, a radial muscle fibre with granular protoplasm and nucleus external.

FIG. 12. A transverse section of the intestine in the 4th somite. Haem. $\times 195$. *cc*, chloragogue cells; *m*, muscular coat; *bs*, blood sinus; *vbs*, ventral enlargement of the sinus; *hm*, muscular walls of the beginning of the heart; *vc*, valve cells; *s*, inter-cellular space; *pp*, peritoneal corpuscles.

FIG. 13. Longitudinal section of a portion of the intestine along the dorsal enlargement of the sinus (6th somite). Alum coch. $\times 195$. The sinus is filled with deeply-stained blood fluid.

FIG. 14. Two transverse sections through the heart. Haem. $\times 230$. *a* is seven sections anterior to *b* in the same series, and is much more contracted. *m*, muscular walls; *n*, muscle nucleus; *pn*, nucleus of peritoneal cell; *vc*, valve cells; *b*, their inter-cellular space.

FIG. 15. A transverse section across a ventral portion of the intestine in the 7th somite. Biondi-Ehrlich. $\times 500$. *c*, ciliated epithelium; *s*, blood sinus; *vs*, its ventral enlargement; *m*, muscular coat; *p*, peritoneum.

EXPLANATION OF PLATE XXX.

FIG. 16. Dorsal view of the ventral ganglia of the head, showing also the anterior termination of the supra-neural blood vessel. $\times 250$. Reduced from a camera lucida drawing of a living specimen.

FIG. 17. Dorsal aspect of the supra-oesophageal ganglion, and termination of the dorsal blood-vessel of the same specimen. $\times 250$. *sg*, principal, and *sg*¹, accessory lobes of the ganglion; *cc*, circum-oesophageal commissure; *va*¹ and *va*², the 1st and 2d vascular arches.

FIG. 18. Transverse section through the ventral nerve cord and ganglia of the 8th somite. Biondi-Ehrlich. $\times 500$. *ln*, *ln*, lateral nerve trunks to body walls; *g*, ganglion cells; *g*¹, larger ganglion cells; *m*, muscular sheath of the nerve cord; *sv*, supra-neural blood vessel.

FIG. 19. The entire atrium of a mature worm, removed from the body and examined in a fresh condition under slight pressure. $\times 195$. Camera lucida outlines, but the histology is purely diagrammatic, and is introduced only to differentiate the various regions. Viewed from the left side. *vd*¹ and *vd*², the anterior and posterior pairs of vasa deferentia; *sv*, glandular spermatic vesicle; *sv*¹, muscular spermatic vesicle; *lm*, longitudinal, and *cm*, circular muscle coats of the penis sheath and copulatory bursa; *ss*, sub-epithelial space; *pe*, penis; *g*, bursal glands; *e*, lining epithelium of bursa.

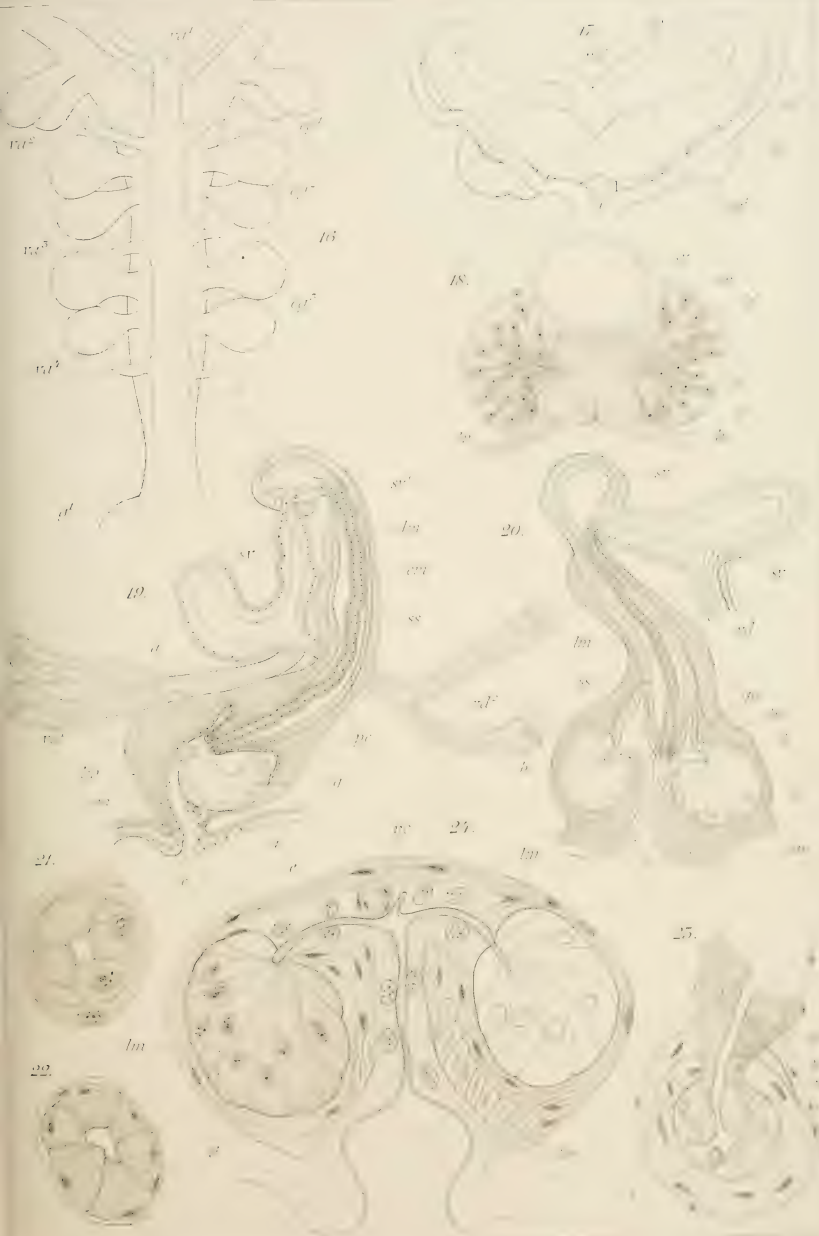
FIG. 20. The same seen from in front; the glandular sperm sac is displaced. Lettering as before.

FIG. 21. Transverse section of a vas deferens, showing the muscle cells and lining epithelium. Haem. $\times 840$.

FIG. 22. Transverse section across the glandular sperm sac. Osmic acid and alum cochineal. $\times 500$.

FIG. 23. Section through the point of union of the glandular and muscular sperm sacs, showing the abrupt change in the character of the epithelium, and the termination of the circular muscle coat. The section cuts the muscular sac transversely and near the side; and the glandular sac longitudinally and almost through its axis. *ge*, glandular epithelium; *e*, non-glandular epithelium; *m*, muscular coat; *p*, peritoneum.

FIG. 24. Vertical transverse section of the copulatory bursa, through the glands. The penis is abnormally retracted. Borax carmine. $\times 500$. *g*, *g*, bursal glands; *e*, epithelium; *lm*, *lm*, longitudinal muscle coat; *tm*, circular muscles; *pe*, penis.



EXPLANATION OF PLATE XXXI.

FIG. 25. Terminal portion of one of the anterior pair of vasa deferentia, from a preparation of the fresh material. $\times 500$. *ml, ml*, limit of muscular layer, beyond which point are seen epithelial cells only; *mn*, nuclei of muscle fibres; *l*, lumen.

FIG. 26. Oblique section across the upper part of the copulatory bursa, approximately through the plane indicated by *a, a* in Fig. 19, and *b, b* in Fig. 20, from a preparation stained in Kleinenberg's haematoxylin. $\times 500$. The right side of the figure reproduces the camera tracings unchanged, the left attempts to depict the appearance of the section. *lm*, longitudinal, and *tm*, circular muscle coats; *be*, epithelial lining of bursa; passing into the glands at two opposite points; *Po*, outer coat of the projecting end of the retracted penis (*Po* is made up of epithelium, circular, and longitudinal muscle fibres just above the plane of the section, but these were obscure in the specimen figured); *ses*, sub-epithelial space; *Pi*, circular muscles of the beginning invagination which forms the penis proper; *Pe*, penis epithelium; *Pl*, lumen.

FIG. 27. Small anterior portion of a longitudinal section through the ovary of a mature individual. Stained in Biondi-Ehrlich. $\times 500$. *S6-7*, muscular septa 6-7.

FIG. 28. Portion of a transverse section through the 7th somite of *B. philadelphicus*, showing the entire length of the left ovipore. Klein. haem. $\times 500$. *o*, a mature ovum; *n*, its nucleus; *oe*, epithelium of ovipore passing into the peritoneum; *cm*, circular muscle fibre, between two of which the external opening is situated; *lm*, longitudinal muscles; *cg*, clitellar glands.

FIG. 29. Reconstruction (from camera tracings of a series of transverse sections) of a spermatheca, seen from behind. Klein. haem. $\times 195$. *ep*, external opening lined by columnar epithelium; *gl*, posterior one of the pair of glands; *slm*, external layer of (longitudinal) muscle fibres, present on proximal portion only; *i*, inner, and *o*, outer limb; *lm*, longitudinal muscles of body walls; *n*, nerve cord.

FIG. 30. Transverse sections of the inner limb of a spermatheca near its blind end. The muscle fibres are here cut obliquely or transversely, owing to their spiral disposition. The appearance of the inner and outer zones in the epithelial cells is shown, and the several nucleoli and indications of an intra-nuclear network. Haem. $\times 840$.

FIG. 31. Omitted.

EXPLANATION OF PLATE XXXII.

FIG. 32. Surface view of a portion of the circular muscle coat (the longitudinal muscle fibres are supposed to have been removed, with the exception of one on each side) of the undivided region of the spermatheca. Haem. $\times 840$.

FIG. 33. Longitudinal section of the anterior nephridial vesicle. Borax carmine. $\times 500$. *c*, the posterior chamber, which would be in communication with the anterior chamber in a section exactly through the median line; *m*, muscular coat, chiefly of circular fibres.

FIG. 34. Terminal portion, with external pore, of posterior nephridium; *en face* and in profile. Free-hand sketch from a living specimen. \times about 275.

FIG. 35. Portion of terminal segment of tubule of the anterior right nephridium, at the point where it passes from the body cavity into the inter-muscular space. Sketch from a living worm. \times about 350. The position of the nuclei midway between the lateral cæca of the lumen, the radial striation of a portion of the tubule, the considerable enlargement in size, and the peculiar looping of the lumen are shown.

FIG. 36. Transverse section across a portion of the anterior loop of the anterior left nephridium, showing four tubules and their investment of enlarged peritoneal cells. The two tubules *n*, *n* have a more dense and radially striated protoplasm than the other pair, which show a reticulate arrangement. *p*, a peritoneal corpuscle. Klein. haem. $\times 500$.

FIG. 37. Oblique section through a single tubule, from the same preparation. $\times 500$.

FIG. 38. Transverse section of a nephridial nodule from the same preparation. $\times 500$. Showing the intra-cellular plexus, and densely granular protoplasm. The two nuclei are located near the enlarged ends of two simple lumens, where they join the plexus. At these points the protoplasm shows radial markings.

FIG. 39. Optical section of three nephridial nodules, with the connecting sections of the simple tubule. Drawn with the aid of a camera lucida from a living specimen. The arrangement of the cilia is purely diagrammatic, and a few of the branches of the plexus were added to the camera tracing. The protoplasm and nuclei have, as nearly as could be copied, the appearance shown. $\times 500$. From the anterior right nephridium. *t*, a short section of one of the loops of a simple tubule.

FIG. 40. Nephrostome of *B. philadelphicus*, drawn from a half-grown living specimen under pressure. $\times 840$. *m*, muscle fibres, to which the nephrostome was attached.



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CONTENTS.

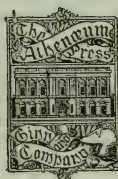
| | |
|---|---|
| The Unrolling of the Universe. Organic Evolution: The Development of Forms of Life, | Are Species Real? Classification, |
| Evolution as a Working Hypothesis, | Application of Theory of Descent to Taxonomy, |
| What Evolution is not, | Application of Theory of Descent to Taxonomy. — Object and Methods of Taxonomic Work, |
| Variety in Life, | Application of Theory of Descent to Taxonomy. — Illustration of the Application of This Method. The Descent of the Lepidoptera, |
| Unity in Life, | Evolution of Plants, |
| Heredity: The Great Conservative Force in Evolution, | Evolution of the Higher Plants, |
| Theories of Heredity, | Spontaneous Generation, |
| The Cell and Heredity, | Man's Place in Nature, |
| Amphimixis, | Evolution in Social Institutions. — Principles, |
| The Meaning of Sex, | Evolution of Social Institutions. — Applications and Illustrations, |
| The Cell Theory, | History of Evolution, |
| The Physical Basis of Heredity, | After Darwin, |
| The Inheritance of Acquired Characters, | Spencer's Formula of Evolution, |
| The Inheritance of Acquired Characters, Continued, | Present Battle-Grounds of Evolution, |
| Significance of Morphology, | The Philosophy of Despair, |
| Ontogeny and Phylogeny, | The Way out of Pessimism, |
| Contemporary Evolution of Man, | Philosophy and Science, |
| The Gastræa Theory of Hæckel, | Religion and Science, |
| The Origin of the Eye, | The Evolution of Religion, |
| The Origin of the Ear, | Evolution and the Bible, |
| The Law of Individuality, | The Fool-Killer and His Mission, |
| The Struggle for Existence, | The Evolution of the Idea of God, |
| Response to External Stimulus, | The Evolution of the Family, |
| Natural Selection, | Saving Time, |
| Natural Selection and Ethics, | The Evolution of the Common Man, |
| Law of Self-Activity, | Books recommended. |
| Law of Mutual Help, or Altruism, | |
| The Origin of Goodness, | |
| Degeneration, | |
| Degeneration in Man, | |
| The Industrial Struggle for Existence, | |
| Isolation as a Factor in Evolution, | |

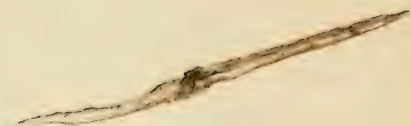
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